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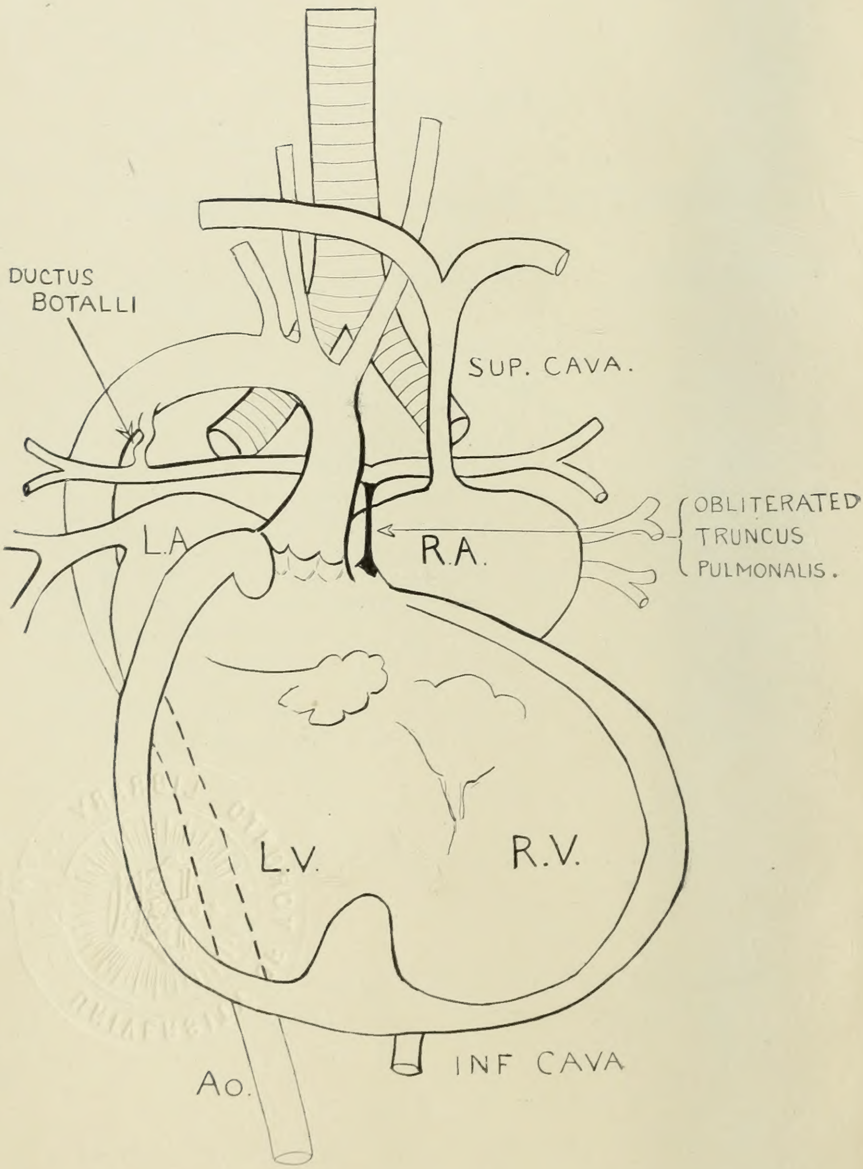
DR. A. B. WADSWORTH, *President.*

COMPLETE TRANSPOSITION OF THE VISCERA, WITH ASSOCIATED DEVELOPMENTAL DEFECT OF THE HEART.

ALWIN M. PAPPENHEIMER, M.D.

Dr. Pappenheimer demonstrated the preserved body of a full-term colored infant, which died an hour after birth, and which showed complete transposition of all the thoracic and ab-


DUCTUS
BOTALLI
SUP. CAVA.
L.A.
R.A.
OBLITERATED
TRUNCUS
PULMONALIS.
L.V.
R.V.
INF CAVA
Ao.

dominal viscera. The left testicle was undescended, the right lay in the scrotal sac.

The case was of interest as illustrating the frequently noted association of developmental defects of the heart with situs inversus. In this instance, in addition to the transposition of the heart itself and the great arterial and venous trunks, the following anomalies were present; (1) single ventricular cavity, with merely a rudimentary septal spur at the apex of the chamber; (2) complete atresia of the conus pulmonalis, which was represented by a thin fibrous cord, about a centimeter in length. This led to patent and well-formed right and left pulmonary arteries. The foramen ovale was widely open, but there was no defect in the interauricular septum. The Ductus Botalli was given off from its usual situation at the isthmus aortæ, but on the right side. It communicated by a wide channel with the patent right pulmonary artery. The conditions found are illustrated by the accompanying diagram.

AN ANALYSIS OF FATTY DEPOSITS IN THE WALLS OF LIVER ABSCESSES.

F. M. HANES, M.D., AND JACOB ROSENBLOOM, M.D.

The liver of a case coming to autopsy at the Presbyterian Hospital, March 7, 1912, was the seat of numerous abscesses whose walls contained large amounts of fatty material of a brilliant yellow color. The color closely resembled that of the adrenal cortex or the corpus luteum. Upon studying the fatty material with the polarizing microscope, it was found to be in large part anisotropic. Upon heating it to about 42 C it became isotropic, returning to the anisotropic, fluid-crystalline phase upon cooling. It stained with Sudan III, but failed to stain when treated by the methods of Smith and Ciaccio. In addition to this anisotropic lipoid material, micro-chemical reactions indicated the presence in lesser quantity of neutral fat and phosphorized fat.

The work of Kaiserling and Orgler, Adami, Aschoff, Kawamura and others leaves little room for doubt that anisotropic fatty material exhibiting the characteristics which I have described in this case is composed of esters of cholesterol. Their conclusions were reached largely from micro-chemical evidence, though in several instances chemical analyses corrobated the micro-chemical findings.

Very little is known concerning the conditions under which lipoids are deposited in tissues. The nature of fatty infiltrations, on the other hand, is fairly well understood. The present instance of lipoid deposits in the walls of liver abscesses seemed to offer a good opportunity of correlating the microscopic and chemical findings and hence Dr. Rosenbloom undertook a complete analysis of the fatty material, using the several very accurate methods which have been introduced within recent years.

Parts of the liver containing no abscesses were analysed for comparison with the analysis of the fatty material in the walls of the abscesses. It was of course impossible to separate the abscess walls completely from the adjacent relatively normal liver; hence to correct this error, the iron content of the fatty material was taken as an indication of the amount of liver tissue included, and upon comparison with the iron content of the undiseased portion, the proper correction was made.

The chemical findings are shown in the following table:

	Normal Liver. Per Cent.	Diseased Liver. Per Cent.
Total fats in dried tissue.....	18.36	72.09
Neutral fats	47.86	2.48
Phosphorized fats	35.12	17.25
Free cholesterol	7.96	2.48
Cholesterol esters	0.63	68.61
Fatty acids	8.43	9.42

Cholesterol esters, it will be observed, form 68% of the total fatty matter present in the walls of the abscesses, and this confirms very strikingly the microscopical findings.

Discussion:

Dr. Ewing: Was this analysis made of the whole liver or only of the pathological portion?

Dr. Hanes: An analysis was made of the whole liver, the normal and pathological portions being first separated as far as possible, and subsequently a correction was made in the figures for the pathological portion on the basis of its iron content.

Dr. Stewart: A great amount of confusion exists in regard to lipoids. A good many dogmatic statements are made in regard to them and one is apt to get the impression that their occurrence is rare. We have often found, in the examination of autopsy material, collections of anisotropic fats which are presumed to be collections of cholesterin esters. Is estimating their relative amounts in the tissue I do not think great reliance should be placed in the present histological methods of examination. A chemical analysis, such as Dr. Hanes has made, is of far more value, in my opinion, than any microscopical examination.

GAS CYSTS OF THE INTESTINE.

WILLIAM THALHIMER, M.D.

History: Patient, I. F., female, aged seventeen years, was admitted November 16, 1912, to the First Surgical Division of Mount Sinai Hospital, after an illness of one day, with pain in the abdomen and distension. Patient looked very sick. The left side of the abdomen was very prominent and the entire abdomen distended. A fluid wave and shifting dullness were present but no tenderness or rigidity. The liver dullness was completely obliterated.

Operation was performed immediately by Dr. A. A. Berg.

When the abdomen was opened, there was a gush of free fluid and gas which had a distinct gastric odor. A collapsed cystic mass, which proved to be a left ovarian cyst, presented, and was removed. A large amount of pus was found in the pelvis. Fluid was discovered flowing from an opening in the posterior wall of the stomach about 5 cm. in diameter, just above the greater curvature. This opening was closed by sutures. The colon was then searched for and was not found in its normal position, but was situated above and in front of the stomach.

Patient was returned to the ward, remained in shock, and died five hours after the operation.

AUTOPSY.

Heart and *Lungs*, normal in all respects.

Abdominal viscera: When re-arranged in the position which they occupied in situ, present a very remarkable appearance. A tremendously distended stomach, which contains 4½ liters of fluid, occupies the entire left half of the abdominal cavity, and the pyloric antrum extends for a short distance to the right of the median line. The stomach is unusual in shape, its lesser curvature being comparatively short and the shape of a V, so that it is sharply angulated on itself. The most dependent portion of the greater curvature reaches as far as the brim of the pelvis. On lifting up the stomach, on its posterior wall just above the omental attachment, there is a surgical suture line about 5 cm. in length parallel to the greater curvature. The stomach seems imperfectly rotated and no definite lesser peritoneal cavity can be made out. The transverse and descending colon do not occupy their usual positions but lie entirely to the right of the enlarged stomach. The greater omentum does not arise from both transverse colon and stomach but arises directly in a single layer from the greater curvature of the stomach, and part of it hangs loosely while the upper portion passes over to that portion of the large intestine which would probably be transverse colon were it normally situated. The stomach wall is greatly thickened and hypertrophied and when the stomach is opened, one finds beneath the suture line on the posterior surface a puckered mass of tissue which has been inverted by the surgeon. No other ulcerations can be found on the mocosa. The pylorus is tightly stenosed and from the gastric side, there is a lip-like or valve-like, somewhat puckered projection around the entire orifice. The tip of the little finger can be passed through the pylorus if considerable force is used. The duodenum beyond seems normal.

Small intestines are moderately dilated and as the ileum is approached, one finds numerous loops of intestines adherent to one another by firm, fibrous adhesions, and situated between the superior surface of the liver and the diaphragm, to both of which structures the loops are also densely adherent. Scattered over these adherent loops of the ileum and situated just beneath the serosa, are numerous thin-walled cyst-like structures. When the ileum is opened, its wall throughout its entire thickness is seen to be honeycombed with these cyst cavities. There are several collections of cysts attached to the mesentery of this portion of the small intestine by pedicles, and several isolated cysts also on pedicles attached to the small intestines along the mesenteric border. These cysts measure from a few millimeters to 2 cm. in diameter. They are tense and smooth and glistening. When incised, they collapse immediately with a small explosion, as though gas were escaping. The above described adhesion of the ileum to the diaphragm has formed behind it an artificial foramen. The loop of ileum which enters the cecum forms the anterior wall of the foramen. The cecum is comparatively undescended and situated slightly above the level of the umbilicus. The ascending colon is consequently about 10 to 12 cm. in length and forms the posterior wall of the artificial foramen and lies immediately upon the first part of the duodenum. There is no transverse colon, for the colon after descending as far as the duodenum, angulates sharply, turns downward and descends, immediately overlying the pylorus. It forms several loops and has a moderately broad mesentery, and from the place where it begins to descend, it has the appearance of the usual sigmoid.

The Liver is slightly smaller than normal. Except for the adhesions on the superior surface of the loops of ileum and additional adhesions to the overlying diaphragm, the liver seems normal. No foramen of Winslow could be found, which further strengthens the opinion that there is no lesser peritoneal cavity.

The Spleen has been pushed up under the diaphragm by the distended cardiac end of the stomach and is flattened out between these two structures. Except for this flattening, the spleen seems normal.

Kidneys are normal.

Uterus and adnexa: Left ovary has been removed, and ligatures are still in place. Peritoneal surfaces are dulled; otherwise negative.

Microscopical Examination: A section of a group of gas cysts hanging from mesentery of ileum by a broad pedicle shows six or seven irregularly rounded cyst-like cavities which vary from 3 to 8 mm. in diameter. These cyst cavities are empty and are lined by an atypical endothelium. This endothelial lining is not complete in all of the cavities but in all of them it is seen in some place or places. In places there is a single layer of fairly normal looking endothelial cells. This, however, as one progresses along the wall, gradually changes in character, becoming cubical, then cylindrical, and finally in places the transformation is seen into fairly large syncytial-like masses of protoplasm, which project from 40 to 50 micra above the basement membrane. The protoplasm of the syncytial masses is very finely granular and pale staining and contains many oval vesicular nuclei, sometimes as many as 12 to 15, which show a tendency to accumulate at the center. The thickness of the cyst walls is from 1/5 to 1/6 of the diameter of the low power field of the microscope. The cyst walls are made up of rather delicate connective tissue, which shows a tendency towards concentric arrangement around the cavities, along with condensation of the cellular structure. With the Van Gieson stain, the cyst walls are seen to be made up entirely of connective tissue. The connective tissue forming the remainder of structure, especially that at the pedicle is rather loose in texture, diffusely infiltrated with round cells and some polymorphonuclear leucocytes. Throughout it, the lymph and blood vessels are very prominent and both types of vessels, especially the lymphatics, show a remarkable proliferative process of their lining endothelium. Many of the small lymphatics are almost completely obliterated by this proliferative process, and in some of the larger lymphatic spaces, endothelial giant cells, containing three or four nuclei, are frequently found. Many of the arteries are also almost obliterated but no multinuclear cells can be seen lining them. The Giemsa stain shows throughout the section, large numbers of irregularly distributed bacilli and a few cocci. These are found everywhere and give the impression of being post mortem invaders.

A longitudinal section of the wall of the ileum shows irregularly shaped cyst-like cavities, corresponding in all respects to those described above, scattered throughout the submucosa, muscularis and serosa, but occurring in greatest numbers in the subserosa. The surrounding connective tissue also shows the same changes in its lymphatics. The entire intestinal wall shows a diffuse round cell infiltration.

The valve-like projection at the pylorus is made up entirely of hypertrophied mucous membrane arranged around a core or stem of smooth muscle tissue. It shows a slight chronic inflammatory reaction. The tissues at its base are normal.

REMARKS.

The pathological condition of gas cysts of the intestines is a comparatively rare one. There are only about thirty cases on record in the literature.

This condition has a definite pathological picture which is in brief, as follows: These gas cysts, with their glistening walls are found situated in any one or all of the coats of the intestines, except the mucosa, i. e. submucosa, muscularis or serosa, though they most commonly occur in the serosa, and may extend down onto the mesentery or project from the latter, attached to it by a broad pedicle. They vary from microscopical size to several centimeters in diameter. They collapse when punctured and the contained gas usually escapes with a hissing or explosive noise. Different observers have reported slightly different analyses of this gas, but most of them agree that it does not differ much from atmospheric air, and only one (Arzt) found that it burned when a match was applied, and this sample contained hydrogen. The cysts are lined entirely or in part by endothelium, though some are lined by bare connective tissue. The cavities are seen to be empty. The endothelial lining forms the most striking part of the picture. In places it is made up entirely of normal flattened endothelial cells with long, narrow dark-staining nuclei. This cell type, however, in most of the cysts, as one proceeds around the periphery, passes through a process of transformation, first cubical, then cylindrical, sometimes several layers of these, and finally there are found large areas of the lining membrane made up of syncytial masses or giant cells of the same type of protoplasm and containing many of the same type of nuclei, sometimes as many as one hundred. These giant cells are the distinctive feature of the microscopical pathology of this process. They are not confined exclusively to the lining of the cysts, but may in some cases be found in the surrounding connective tissue, where they seem to occupy the lymph spaces or capillaries. One observer (Finney) reports what seemed to be vacuoles made up of gas within these giant endothelial cells.

Bacteriological studies have shown in a number of cases that the cysts are sterile, and in other cases various types of organisms have been recovered, the discrepancy indicating that the etiology is not a bacterial one. Sections stained for bacteria have shown the same irregular results; protozoa have never been found.

Similar cysts have been found in swine, usually in the mesentery, and containing the same giant cells. A Gram-negative bacillus, however, is always associated in these animals with this condition. The disease has never been reproduced experimentally with this organism, but many of the experimental animals die after intraperitoneal inoculation.

The two discriminating factors between ante-mortem and post-mortem gas cysts of the intestines are the presence of giant cells and absence of gas-forming organisms in the former, and just the reverse in the latter.

A striking feature of this pathological condition is, that, in most cases, the gas cysts cause no intestinal symptoms but are associated with some other disease of the gastro-intestinal tract, usually some form of partial obstruction. It would seem that the case reported by Blatteis is the only one in the literature where the gas cysts themselves gave rise to intestinal symptoms.

The case above described shows all the characteristics of this pathological process and is associated with the condition of a tremendous dilatation of the stomach, due to benign pyloric obstruction. In addition there are marked adhesions at the site of the gas cysts between the intestine and diaphragm and superior surface of the liver. This may have been caused by the gas cysts themselves. It seems rather remarkable that neither the pyloric stenosis or intestinal adhesions gave rise to symptoms.

The etiology of this condition still remains undiscovered. Some authors consider it a congenital anomaly. Others think that it arises through infection of the intestinal wall by various gas-producing bacteria entering through minute lesions to the mucosa. As minute injuries to the mucosa must be a comparatively common occurrence and the condition of intestinal gas cysts is a comparatively rare one, the latter can hardly be considered to arise from the former.

The origin of this process from a specific bacterium is not borne out by the bacteriological studies. One author (Finney) considers these gas cysts to be true tumor formations, by cells having the capacity to secrete gas, similar to the cells lining the air bladders of fishes. This is an attractive theory but there is not as yet sufficient proof to bear it out.

By far the most satisfactory explanation is that of Arzt, that there is primarily a more or less localized proliferative process of the endothelium of the lymphatics, the cause being unknown. This proliferation goes on to complete obstruction with damming back of lymph, and then gas collects in the lymphatics, its origin being unknown. Next, the formation of gas cysts and the giant cells form as irritation giant cells from the gas. Even with this explanation, we do not know what causes the lympathic endothelial proliferation or the gas formation. This must be left for future investigation. One thing seems quite definite, and that is, that the obstructive endothelial proliferation of lymphatics has an important etiological bearing on the formation of the gas cysts, which seem to arise by distension of the lympathic spaces or vessels.

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Arzt, L., Zur Kenntniss der Pneumatosis Cystoides des Menschen, *Frankfurt Zeit. für Path.*, 1911, vi, 85.

Finney, J. M. T., Gas Cysts of the Intestines, *Jour. Amer. Med. Assc.*, 1908, li, 1291.

Blatteis, S. R., An Unusual Lesion of the Large Intestine, *Proc. N. Y. Path. Soc.*, 1910, x, 102.

Discussion:

Dr. MacCallum: There was a case reported several years ago by a Western doctor in which the cysts had thin shining walls. It was thought that this case might have been due to a gas bacillus infection, but there was no connection between the thinly lined cysts and the intestinal wall.

Dr. Norris: There is a recent article on this subject in Virchow's Archives by Poll, who maintains that the microscopic findings in gas cysts are not typical. Many cases reported as gas cysts are denied by him.

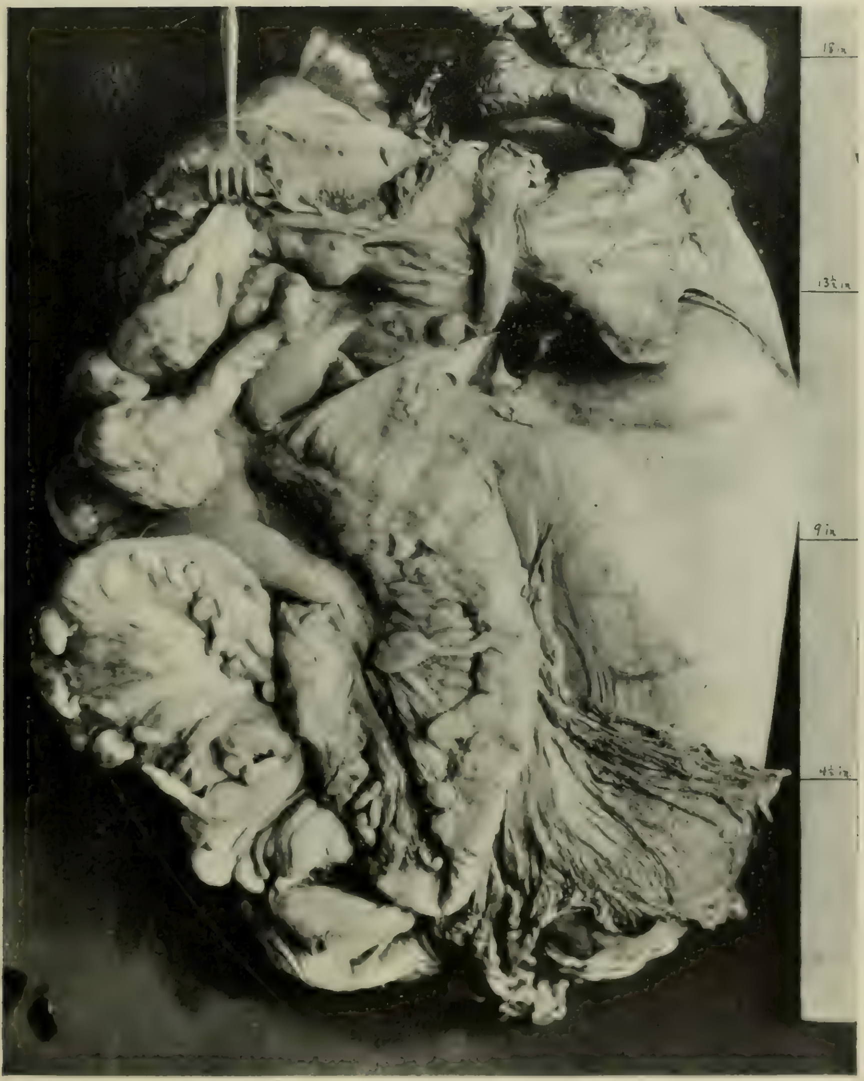

FIGURE I.

Shows the gas cysts over the ileum, and the abnormal position of the colon.

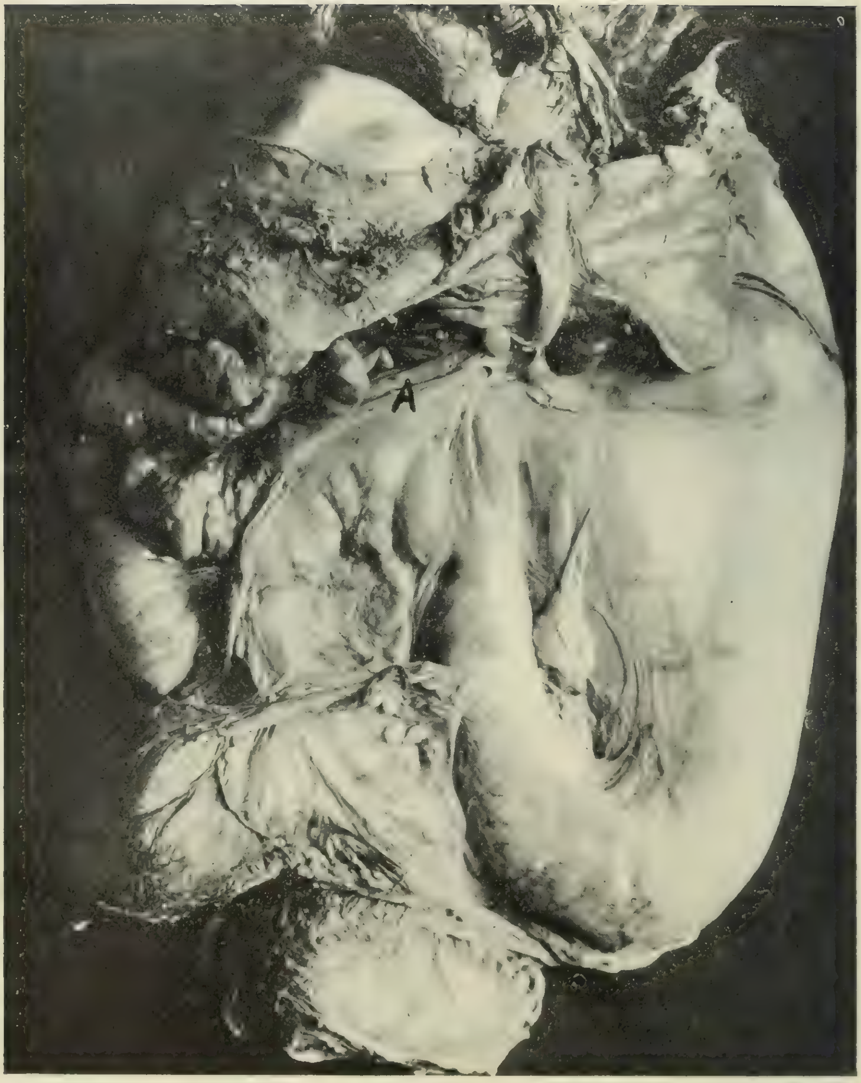

FIGURE II.

Shows the unusual shape of the dilated stomach. The diaphragm is seen overlying the liver and adherent to it. The adhesion of the ileum to the liver and diaphragm is shown. Just above 'A' an artificial foramen formed by this adhesion is found.

A CASE OF OCCLUSION OF THE LEFT CORONARY ARTERY BY A PEDUNCULATED VEGETATION OF THE AORTIC VALVE.

ALBERT LAMB, M.D.

While instances of coronary thrombosis are fairly common, cases of true embolism of these arteries are sufficiently rare to arouse considerable interest. The effects of the two conditions are so similar that they are generally considered together.

Experimentally, much work has been done on the question of the effect of coronary occlusion. The earlier researches, which culminated with Cohnheim's experiments in the early eighties of the past century, demonstrated that the coronaries were end arteries and that the occlusion of a large branch was sufficient to cause sudden death. Since then experimental work has tended to refute these views. It would require too much time to discuss these experiments in detail. It is sufficient to note that by the work of Jamin and Merkel, of Amenomiya and of Spalteholz and Hirsh, to mention only a few of those who have given the subject their attention, the coronaries have been shown to have visible and functionating anastamoses and that the occlusion of one of the larger branches does not necessarily result in sudden death, or, indeed, prove fatal at all.

Clinically, the same conclusion has been reached. For example, Pagenstecher ligated the descending branch of the left coronary in an accident case. The patient survived five days. Fraenkel had much the same experience. West, Chiari, Merkel, Dock, Recklinhausen, Galli, Huber, Aschoff, Huchard, Herrick, Kidd, and others have all cited cases showing extensive disease of the heart muscle due to thrombotic occlusion of a coronary artery where death was postponed for varying lengths of time or did not occur until much later, and then from some other cause.

Herrick, in his recent article, expresses the present view of the results of coronary occlusion as follows:

"While sudden death often does occur, yet at times it is postponed for several hours or even days, and in some instances a complete, i. e. functionally complete, recovery ensues."

The cases of true embolism reported are few in number. Chiari, Oestreich, Virchow, Rolleston, Hektoen, Welch, MacCallum and Thorel have described instances.

The literature is well covered by Welch in his articles in *Albutt's System of Medicine,* by Thorel (*Ergebnisse der Pathologie,* Lubarsch & Ostertag, 1903) and by Herrick (*Jour. A. M. A.,* Vol. lix, No. 23, December 7, 1912.)

The case which I have to present to-night was in a negro ballplayer, thirty-five years old, who was admitted to the medical service of the Presbyterian Hospital on October 30, 1912. I am indebted to Dr. Janeway for permission to report the case. As a child the patient had rheumatism. Up to two months before admission he had weighed 190 pounds. He had lost gradually until on admission he weighed 157 pounds. This loss of weight, together with loss of strength, constituted his sole trouble until a few days before entering the hospital, when he began to have pain in the right side of his chest, posteriorly.

On admission he was found to be a large, well-built negro, not dyspnoeic or cyanotic. No petechiæ could be found. The lungs were normal except for slight diminution of the breath sounds. The apex impulse of the heart could not be seen or felt. The heart sounds were loudest in the fifth intercostal space, 10 cm. to the left of the mid line The sounds were of fair quality and the action regular. At the apex the first sound was accompanied by a blowing murmur transmitted to the left axilla and upwards along the sternum to the pulmonary area, gradually becoming less marked away from the apex. Just to the left of the sternum, in the third and fourth intercostal spaces, there was also a soft diastolic murmur.

A blood culture taken on admission turned out later to show a growth of what were considered endocarditis cocci.

On the day after admission the patient was perfectly comfortable. At 8 P. M. he was seen and was all right. Ten minutes later he was moaning and complaining of pain in his chest, over the precordium. He was pale, perspiring profusely, and had a weak pulse. Vomited once. Adrenalin was given. Patient was at this time extremely pale, almost pulseless and complaining of agonizing pain in the back. He was given more stimulation with morphine and a saline infusion was started. It was noted that the vein when exposed was collapsed and practically bloodless. After about 500 cc. of the infusion had been given the patient stopped breathing. His breathing had not been rapid or labored, but in the last half minute it became gasping with increasing intervals.

Autopsy, November 1, 1912. A large framed man, showing considerable loss of weight. No petechiæ found. There was about 100 ccm. of clear fluid in the left chest. The lungs were in full inflation, showing the markings of the ribs over their upper surfaces. Both lungs showed congestion and oedema. The pericardium contained about 100 cc. of clear fluid. The pericardial surfaces were smooth and glistening. Before removing the organs the pulmonary artery was opened and traced to its larger branches without discovering any obstruction.

The heart was not enlarged. The right side presented no abnormalities. The left auricle, ventricle and mitral valve showed no change. No thrombi or post-mortem clots were found in any of the heart cavities. The aortic valve showed the left cusp divided by the incision. The posterior and right cusps were fused into one. The former line of separation could be seen in the Sinus of Valsalva. The opening of the right coronary artery could be seen behind the right portion of this fused cusp. There was a slight amount of yellow atheroma about its mouth but it did not encroach upon the lumen. This large cusp was covered with soft, irregular nodular vegetations, with which was mixed some fresh clot. The vegetations were confined almost entirely to the ventricular surface of the valve, the other surface showing only a few small ones. The lesion as seen upon the left cusp was more destructive. A portion near the right cusp showed no vegetations but was stiff and sclerotic. The rest of the cusp was partially destroyed and replaced by large, irregular, soft masses of vegetation of dirty pink color. Near the line of junction with the posterior cusp, the vegetations formed a small reddish mass and encroached upon the Sinus of Valsalva. From the summit of this mass there arose a thin pedicle of the same vegetation tissue. It was ½ mm. in diameter and about 6 mm. long. At the end away from the valve it ended in another small nodular vegetation which completely plugged the mouth of the left coronary artery. The opening of the coronary was completely occluded, but the vegetation only extended a short distance into the lumen, the rest of the plug being thrombotic in nature. The plug was pulled out and found to be non-adherent and the wall of the artery smooth. The plug was 1 cm. long, forming a complete cast of the lumen of the vessel. The lower end was branched, fitting into the descending and circumflex branches. There was no change in the heart muscle supplied by these branches.

Infarcts were found in the spleen and kidney.

From the appearance of the plug when pulled out of the coronary it would seem fairly certain that this pedunculated vegetation had swung free in the blood stream for some time and that it had suddenly been swept into the mouth of the left coronary just before the patient's death.

Discussion:

Dr. MacCallum: I reported one case in which death followed at once upon the occlusion of the anterior branch of the left coronary artery by a loose vegetation. There was no change in the heart wall, but in other cases observed sufficient time had elapsed before death for the development of an infarct of the wall. In one such instance I found the wall of the ventricle ruptured and the pericardium full of blood.

VARIOUS FORMS OF CALCIFICATION AND OSSIFICATION. OVARY (5 CASES) FALLOPIAN TUBE, (1 CASE), LUNG (1 CASE), OSSIFIED EPITHELIOMA (1 CASE), CALCIFIED HERNIAL SAC (1 CASE), MYOSITIS OSSIFICANS (1 CASE).

ELI MOSCHCOWITZ, M.D.

Ossification in Ovary.

Case 1: Ovary removed for chronic ovaritis. Situated beneath the capsule of the ovary is a corpus luteum just entering the terminal phase of its evolution. The corpus luteum is converted into a scar-like mass, a few cellular elements being visible in the peripheral portions. Scattered through the outer zone are a large number of small round foci of lime deposits, discrete, and sharply defined from the surrounding tissue.

Summary: Fecal deposits of lime in a healing corpus luteum.

Case 2: Ovary removed at autopsy from a patient, aged sixty, suffering from chronic nephritis. There is a mass in the center of the ovary 5x3x2 mm., of ivory hardness. The surface is convoluted. The calcified mass is situated in the center of a corpus albicans, a thin zone of hyaline connective tissue surrounding it. Microscopically the mass consists of a homogeneous mass of lime imbedded in a matrix of hyaline connective tissue derived from the corpus albicans. The edge is sharply circumscribed from the surrounding connective tissue, wavy, and finely spiculated. Within the calcified area are clefts which are manifestly artefacts.

Summary: Focal deposits of lime in a healing corpus luteum. bicans.

Case 3: Ovary removed in the course of a hysterectomy for fibroids. Patient aged forty. A hard calcareous mass, 8x5x4 mm., with convoluted surface was found just beneath the capsule of the ovary situated in the central portion of a corpus albicans. The contour is wavy, also slightly spiculated, but differs from the preceding specimen in that there are numerous fairly deep indentations on the surface, which, in many places, can be shown to communicate with irregular cavities within the calcified nodule. On close examination of these indentations, it is seen that they contain a fine fibrillar connective tissue, a number of young fibroblasts and many red cells, but no blood vessels. These same elements are also found in the cavities within the nodule.

Summary: A calcified nodule of the ovary revealing indentations and cavities containing blood and young connective tissue.

Case 4: Case of double salpingo-oöphorectomy for tuberculosis of the tube in a woman aged thirty-three.

Calcareous mass in the center of the right ovary, 1.7x1.3x1.1 cm. On removal of the mass only a shell of ovary, 2 mm. thick, is left. The surface has a convoluted, brain-like appearance. On section, the outer third of this mass consists of lime similar in morphology to case 3. We find also the same indentations and cavities noted in case 3. A further change, however, is noted in the increase of fibroblasts in these situations, and their arrangement along the periphery of the cavity in an osteoblastic layer. Coincident with this we see absorption of lime around the margins of these cavities with penetration of the osteoblasts for varying distances. Furthermore, we already note around some of these cavities, the conversion of this osteoblastic layer into true bone. The red blood cells no longer lie free but in fine delicate blood vessels. The central portion of this mass consists of true bone; with lamellar bone cells and Haversian canals containing well formed blood vessels, fibrous tissue and fat.

Summary: Calcareous nodule in ovary, showing osteoblastic process in the periphery and ossification within, spreading eccentrically.

Case 5: Specimen of Dr. W. C. Clarke, Pathologist of the General Memorial Hospital. Mass the size of a pecan nut in the center of the ovary. Same conformation and morphology as preceding except that the changes are more advanced.

Summary: Same as preceding.

Epicritical Remarks.

The formation of bone within the ovary is evidently an eccentric process proceeding from the center of the calcified area towards the periphery. In all cases there is preliminary calcification of a corpus albicans. Blood sinuses penetrate this area from the periphery and form cavities within. These sinuses bring with them fibroblasts which later assume the function of osteoblasts. Coincidentally there is lime absorption and a conversion of the osteoblasts into bone. The blood sinuses become converted into blood vessels. Each primitive indentation and cavity in this manner becomes a Haversian canal, around which the new bone is formed.

The process is entirely analagous to ossification with the exception that instead of cartliage the underlying matrix is calcified hyaline connective tissue.

OSSIFICATION OF THE FALLOPIAN TUBE.

Case 6: Fallopian tube removed from the same patient as case 3. Both tubes were adherent. The left tube is distorted and its outer half dilated. At the isthmus there is a typical nodule of "Salpingitis Nodosa." The walls of the tube are greatly thickened near the fimbriated end and lying between the mucosa and peritoneal surface is a circular plaque of bony consistency surrounding the lumen of the tube.

Microscopical Examination: The left tube has been converted into a fibro-adenomatous structure. The fibrous tissue is dense hyaline, and resembles a corpus albicans in morphology. The calcareous plaque shows in part true bone and in part merely lime infiltrations of the hyaline connective tissue. The same indentations and cavities as in cases 4 and 5 are noted here; also the osteoblastic processes and their transformation into osseous tissue.

The right Fallopian tube was the seat of a typical tuberculous salpingitis.

OSSIFICATION IN THE LUNG.

Case 7: Tubercle at the apex, 6x3 mm., found post-mortem in a man fifty-one years of age, who died of chronic nephritis. There is a central core of amorphous granular lime, consisting of a number of irregular nodules, one larger and four or five smaller ones. Each nodule is firmly enclosed in a fibrous capsule, which is in part calcified and in part reveals true bony lamellæ. The lime or bone in this capsule is found adjacent to the calcified core; the peripheral portion is fibrous, resembling the periosteum. On the outer aspect of the bone are cells resembling osteoblasts. The whole area is surrounded by an elliptical thin capsule of bone covered on its outer side by a periosteal-like layer of fibrous tissue which connects the bone to the surrounding lung tissue. On the outer aspect of the bone are cells resembling osteoblasts. On the inner aspect trabeculæ of either dense fibrous tissue or bone pass inward, uniting with the osseo-calcareous capsules surrounding each calcareous nodule. Between these two there is marrow-like structure consisting of fibrous tissue, fat, blood vessels and lymphoid cells.

Summary: A cheesy tubercule of the lung, made up of a number of smaller tubercles, which has calcified. Each of the component tubercles is surrounded by a fibrous capsule which has in turn calcified and in part ossified. The whole mass is surrounded by a bony capsule. Between the two capsules there has developed a marrow-like cavity.

Epicritical Remarks.

The process is on too small a scale to permit of definite conclusions being drawn, but it appears that the process of ossification here resembles rather the periosteal rather than the endochondral type.

OSSIFIED EPITHELIOMA

Case 8. Tumor of the abdominal wall of twenty years' standing in a patient aged sixty. The mass was 2.5 cm. long. It was ovoid in shape and at its thickest part measured 1.5 cm. in width and 8 cm. in depth. The tumor was of bony hardness and sharply circumscribed from the surrounding tissue. The tumor was entirely subcutaneous and nowhere showed any connection with the overlying skin epithelium. The main bulk of the tumor consists of nests of epithelial cells, which have undergone keratohyalin degeneration. The cells show regularity in size and arrangement and morphologically conform to the basal cell type. The majority of the epithelial nests are surrounded by a narrow zone of osseous tissue, composed of typical lamellæ, and Haversian canals. On the external aspect of these bony capsules there are cells of the osteoblastic type. These cells are also present along the periphery of the Haversian canals. The osseous tissue is in direct apposition to the outer layer of epithelial cells. Osteoblasts are never encountered in this region. Other nests are surrounded by a dense hyalin connective tissue, parts of which have been converted into osseous tissue. The osseous and connective tissue alveoli intercommunicate, forming a labyrinth of trabeculæ. On the other hand some nests are not surrounded by any differentiated form of connective tissue, but merely by the surrounding subcutaneous fibrous tissue. Some of these nests, especially at the periphery of the tumor are enclosed by foreign body giant cells. About the entire periphery of the new growth, beyond the limits of the epithelial cells, are small irregular deposits of amorphous lime in the connective tissue and a broad zone of round and plasma cells.

Summary: Subcutaneous non-malignant epithelioma of the abdominal wall, the epithelial alveoli of which are enclosed in bony capsules.

Epicritical Remarks.

The pathogenesis of this specimen is quite clear. It represents the healing process of a non-malignant subcutaneous epithelioma, probably of sebaceous gland origin. The beginning of the healing process was a keratohyalin degeneration of the epithelial cells. In the surrounding connective tissue a deposit of lime occurred. Some of the surrounding connective tissue cells assumed the function of osteoblasts and formed bone. Coincidentally the surrounding blood vessels penetrated the ossifying zone and formed marrow spaces. Here again we must assume a process analogous to membranous ossification.

CALCIFICATION OF HERNIAL SAC.

Case 9: The patient was a man forty-four years of age, who had a hernia of three years' duration. Strangulation occurred and the sac was removed at operation.

The entire sac was studded with round, flattened masses, varying in size from a split pea to a bean. The masses were in the connective tissue wall and were covered by peritoneum.

The microscopic picture shows merely a deposition of lime in a dense hyalin subperitoneal connective tissue. There is not the slightest manifestation of ossification.

General Conclusions.

What general observations can be drawn from the various types of calcification and ossification?

As a rule pathological calcification occurs in the human body within dead tissues or tissues of low vitality. The most familiar examples of calcification in dead tissues is seen in tuberculous foci, atheroma of blood vessels or heart valves, old infarcts, necrosed fibromyomata and in the interior of sebaceous cysts. The most familiar examples of calcification occurring in tissues of low vitailty are scars of whatever origin, whether from trauma or operation; the healing of corpora lutea, or of a profound chronic inflammation, as in our case of the tube. The occurrence of bony deposits in tendons, as the result of chronic irritation can be ascribed to the same cause.

The cases shown exemplify lesions occuring in both types of tissue. We have shown five cases of calcification on the ovary; one of a tube which has been transformed by chronic inflammation into a scar-like structure; one of a manifestly healed cheesy tubercle of the lung; one of an inactive and apparently healed epithelioma of sebaceous gland origin and one of a hernial sac, the connective tissue coat of which has undergone dense fibrous scar-like transformation.

In five of the nine cases true bone associated with calcareous deposit is noted. That this is not an unusual association is indicated by the observations of Wells, Poschorisky, Lick, Buerger, Oppenheimer, Harvey and others. Poschorisky, for instance, found bone in all but four of twenty-nine cases of calcification in the eye. He found bone in seventeen of twenty-eight calcified nodules in the lungs. In all my cases and those hitherto reported the process of calcification precedes that of ossification. Ossification without preliminary calcification is never found, and without calcification there cannot occur ossification.

The pathogenesis of the various types of ossification in tissue has puzzled observers, as the various theories testify. In the ovary, for instance, the osseous tumors are held by many to be true blastomas, or teratomas; others merely ascribe the process to a metaplasia, without indicating specifically the nature of this conversion.

It seems to be quite clear, as I have already indicated, that the deposition of bone within calcareous nodules represents two different processes analogous to the two main types of ossification occurring normally in the human frame, the endochondral and the periosteal. The main difference between the normal methods of ossification and those represented in our specimens is, that instead of cartilage the underlying matrix is calcified fibrous tissue.

Vascular supply apparently plays a large role in the process of ossification. This explains why calcareous concretions within hollow organs, for instance, the appendix, never reveal bone formation.

EXTRATESTICULAR TERATOMA TESTIS.

JAMES EWING, M.D.

One of the obscure features of certain teratomas of the testis is the presence of a peculiar type of large cell malignant tumor growth which often accompanies the new adult tissues of the teratoma. This malignant tumor has the structure of the common large round cell tumor of the testis, often called sarcoma, and for which, on account of its peculiar structure I have suggested the term embryonal carcinoma with lymphoid stroma (*Surgery, Gynecology and Obstetrics,* 1911, p. 230).

The exact origin of this tumor is not fully determined, although its epithelial nature is now generally admitted. Chevassu believes that its origin is from the spermatoblasts, and he employs the term "seminome." While there are several considerations which support the conclusion of Chevassu, a study of many of these cases has led me to think that this tumor is a part of a teratoma and not derived from adult spermatoblasts or from any portion of the normal testis. This view adopts the principle of the predominance of one element in a teratoma, established by L. Pick, and others, and opens the way for the acceptance of a single origin for all the common tumors of the testis. The occasional occurrence of teratomas with embryonal carcinoma outside of the tunica albuginea but attached to the testis, is a fact which favors the embryonal and teratomatous origin of the large cell tumor of the testis. Koslowski (*Virch Archiv.,* vol. 148, p. 36), has reported such a case of extratesticular teratoma with embryonal carcinoma.

I have recently encountered a teratoid tumor with portions of the characteristic embryonal carcinoma, sharply separated from the body of the testis and wish to record the case as further evidence in this field.

The patient was a healthy subject, thirty years of age, who had noticed a swelling of the testis for one year before examination. He was sent to Dr. F. C. Edgerton (with a diagnosis of tuberculous epididymitis) who removed the growth under the impression that it was either a gumma or a tumor. The Wassermann test was negative.

At operation the tumor was found to be well circumscribed, about 6x8 cm., in dimensions, and closely incorporated with the epididymis and neighboring tunica albuginea, from which, however, it was readily shelled out, much as the prostate shells out of its capsule. The testis, which was apparently uninjured, was not removed, and there has been no recurrence since the operation, now six months ago.

The specimen, for which I am indebted to Dr. Edgerton, was composed of a rather firm lobulated mass, containing four somewhat separate portions, one of which was rather soft and ill defined. No portion of the testis or epididymis could be discovered. On section, three of the tumor lobules present the usual features of teratoma of adult type, including epidermoid cysts, islands of cartilage, masses of smooth muscle tissue, alveoli and cysts lined by high cuboidal or columnar epithelium, several sympathetic ganglia, and other unidentified traces of fetal mimicry. Some of the epithelial structures showed carcinomatous changes of peculiar types, differing from the embryonal carcinoma with lymphoid stroma. The soft irregular mass of tissue was composed exclusively of embryonal carcinoma with lymphoid stroma, and its occurrence in a tumor so well separated from the body of the testis seems to show that this structure does not originate from the tubules of the testis, but from the original cell or cell group giving rise to the teratoma.

This conclusion, however, cannot be regarded as absolutely certain, since it is conceivable that the whole tumor arose from misplaced portions of testicular tissue in the tunica albuginea and epididymis.

TERATOMA TESTIS: CHORIOMA OF LIVER AND LUNGS: TRIDERMAL METASTASES IN LYMPH NODES.

JAMES EWING, M.D.

Clinical History: Patient, J. N., aged thirty-two years. First noticed shortness of breath and cough, followed soon by a tumor in right lower region of neck and feeling of fullness in chest about October 1, 1912. A troublesome cough soon became established and was attended by increasing difficulty in breathing. The glands of the lower left side of neck soon became enlarged and the patient rapidly lost strength with increasing dysponea. On admission to the General Memorial Hospital, November 21, 1912, he was markedly cyanosed and suffering chiefly from dyspnoea.

He was of muscular build and well nourished, pulse 120, temperature normal. On each side of the neck, just above the clavicle and behind sternomastoids there were present, nodular, firm, painless, immovable swellings, suggesting lobulated tumors rather than a mass of lymph nodes. On the left side these extended above lower border of larynx. The cervical tumors are continuous with an area of flatness in the mediastinum.

Resonance over both chests is markedly impaired in patches and there are rales all over both lungs. Through the muscular abdominal wall irregular resisting masses can be detected along the vertebral column.

Axillary and inguinal nodes, normal.

The dyspnoea and cyanosis persisted unchanged and the patient died suddenly on December 4, 1912.

Autopsy.

Body of a robust adult male. Slightly anemic. Breasts not notably enlarged. Above both clavicles are several rounded tumors beneath skin.

Heart: Normal.

Lungs: Voluminous; the seat of many tumors. Trachea and bronchi extremely compressed. Two types of nodules are seen: (1) Very prominent nodules projecting above the pleura 3-5 mm., hemorrhagic centers, gray peripheries .5-2 cm. in diameter; typical chorioma. (2) Equally prominent, cystic tumors, firm white elastic, which prove to be cysts of various types lying in cellular connective tissue.

Pulmonary parenchyma emphysematous and congested. Bronchial nodes much enlarged. Region of thymus and mediastium occupied posteriorly by one large mass 10 cm. in diameter, and several smaller masses 3-5 cm. Several cystic isolated tumors in the lower cervical lymph nodes. None in axillæ.

Abdominal Cavity, contains considerable fluid blood with much fibrin covering the omentum. Blood emanates from a soft tumor, 7 cm. in diameter, in the left lobe of the liver.

Liver: Size, consistence and markings normal except for this tumor, which on section is crumbly, papillary and closely resembles a two months' chorion.

Kidneys: One small solid tumor in the left renal cortex; kidneys otherwise normal. Adrenals, normal.

Gastro-intestinal Tract: Normal.

Lymph Nodes at coeliac axis and along the lumbar vertebrae much enlarged, each 2 to 6 cm., discreet, solid and cystic, whitish or mucinous on section.

Left Testicle, slightly enlarged by the presence of many hard pea-sized nodules at upper pole and along the cord up to the inguinal ring.

Section shows small, hard cystic structures beginning at rete extending up 5 cm. Testicle contains several small opaque grayish massees.

Inguinal Glands not enlarged.

Diagnosis: Tératoma Testisis with Metastases; Chorioma of Liver and Lungs; Acute Anemia.

There are several distinct varieties of structure represented in the tumors: (1) Typical chorioma, of which there is a small nodule in rete testis, one large tumor in the liver and many in the lungs; (2) Hard cystic growths in the testis, cord, lymph nodes, which are simple epidermoid cysts surrounded by cellular connective tissue, or glandular cysts with cuboidal or columnar epithelium surrounded by a very cellular connective tissue; (3) One small mass of embryonal carcinoma with lymphoid stroma in testis; (4) Adult glandular structures, adeno-carcinoma, scanty islands of cartilage and various other abortive mesoblastic and epiblastic structures in abdominal and thoracic lymph nodes. Many masses show curious epithelial carcinomatous foci resembling renal glomeruli; no brain tissue or striated muscle is seen, but embryonal smooth muscle is present. Some of the mediastinal tumors contain structures resembling embryonal enamel organs. Examination of blood and lymph paths shows that the chorioma passed along the blood vessels, while the epithelial elements of dermoid cysts, etc., spread through the lymph paths.

Epicritical Remarks. This remarkable case emphasizes, first, the necessity of careful palpation of the testis in male subjects presenting tumors of any sort in abdomen or chest. The first definite symptom noted by this patient was the appearance of a swelling in the neck. The primary tumor in the testis remained very small, was palpable only around the epididymis, suggested merely a thrombosed varicocele, and was overlooked during life. The duration of the disease was very short, although probably much exceeding the period of ten weeks covered in the ascertained history.

This tumor follows the rule by originating in the rete testis and extending by the lymphatics for most of the elements, by the blood vessels exclusively for the chorioma.

The chorioma appeared in one small area in the rete testis and spermatic vein, many masses in lungs, one in kidney, and one in liver. The hepatic tumor is of special interest as it closely resembled chorionic tissue in the gross and presented the typical structure of chorio-carcinoma. Langhans cells and syncytium were clearly distinguishable, but very hypertrophic and with nuclei hyperchromatic. Apparently when true chorioma occurs in the liver its structure is unmistakable. In Warthin's case the same typical structure was maintained. These observations do not favor the tendency to interpret as chorioma many hemorrhagic tumors of the liver which are probably carcinomas. Curiously enough the hepatic tumor which resembled an early chorion or placenta was the source of a fatal hemorrhage. Warthin and Garborini in their cases observed hypertrophy of breasts and secretion of colostrum. The conception of pseudogestation in the male thus appears not inapt.

An anatomical feature of interest concerns the age of the different histological types of tumors present in this case. Some authors have spoken of the relatively adult tissues of such tumors as coetaneous with those of the host, while the cellular embryonal structures have been interpreted as representing a progeny of the host. It has been held that different modes of origin of teratomas of the sex glands are indicated by these differences in structure. Yet in the present case there seems to be every reason to assume that all the tumors were of the same age and origin, and that the very cellular structures of the embroyonal carcinoma in the testis, and chorioma in liver did not outstrip the mesoblastic and epithelial tumors in lymph nodes and lungs.

Small areas of embryonal carcinoma with lymphoid stroma, the common mixed cell tumor of the testis, were found in the rete and secondarily in the body of the testis. In these positions they furnish little evidence regarding the exact origin of this portion of the tumor, but the assumption may be maintained that it was an integral part of the teratoma.

Discussion:

DR. FRANK: I am very much interested in both of the cases which Dr. Ewing has presented, the second one particularly is a very rare one. In 1906 I reported a case which I thought at the time was a mediastinal teratoma, although owing to the technique of the autopsy there was doubt as to whether the testicles were normal. The mediastinal part showed teratomatous portions; and in the liver and lungs there were metastases which were typically chorio-epitheliomatous. Several years have passed since I have reported this case, and during that time I have been paying more attention to physiology than pathology; hence I feel less positive of the morphological criteria used to classify tissues, and more inclined to credit much of the fetal mimicry to environment and to the stimuli producing tumor growth. It seems to me now that the place of metastasis determines largely the form of the embryonal tissue which develops at the new site, and I incline toward the view of those investigators who believe that the liver has some influence in making these embryonal cells assume the syncytial form.

A great deal of weight is attached to the papillary appearance of these tumors. Again, certain surroundings favor papillary shapes, (as for instance, intravascular growths), and, therefore, I think that we are again led to exaggerate the specific value of such formations.

Dr. Ewing divides chorio-epithelioma into chorio-carcinoma, chorio-adenoma and syncytial forms. If such classification will help in diagnosis it is of value, but I cannot agree to Dr. Ewing's classification until careful analysis of a very large number of cases of chorio-epithelioma, in which the history, subsequent course and morphological type of the tumor have been thoroughly investigated, substantiates his views. Until then I shall continue to feel that Marchand, Schmorl, Pick and others are right when they claim that the morphology is no guide in making a prognosis.

Dr. MacCallum: I should like to ask Dr. Ewing if his second case showed any obvious growth into the blood vessels.

Dr. Ewing: There was an infiltration into the blood vessels at the brim of the pelvis which probably grew from below.

I am interested in Dr. Frank's remarks and think his argument is well founded regarding the limitations of fetal mimicry in teratomas. If one does not use caution the most bizarre constructions may be put on many of these appearances. I feel, however, that it is best to freely admit such obvious facts as are sometimes seen in these cases. The metastases may go where they please but the cells reveal the natural tendencies of embryonal tissues wherever they lodge. The different tumors in the liver and lung showed no variation in structure and were identical with those in the rete testis. On the whole I have not been impressed with the influence which the soil plays in determining the behavior of metastases of teratomas, and although I recognize the soundness of Dr. Frank's argument it does not seem to apply in this instance.

REPORT OF A CASE OF ARTERIOVENOUS ANEURISM OF THE SUPERFICIAL FEMORAL VESSELS.

A. B. EISENBREY, M.D.

(Resident Pathologist, St. Luke's Hospital.)

The patient, a physician, aged sixty-three years, was admitted to St. Luke's Hospital in December, 1912, on the service of Dr. Hollis. He complained of shortness of breath and presented symptoms of cardiac insufficiency. The history of his disability extended over a period of two years. He gave no history of ailments referable to the genito-urinary, respiratory, or gastro-intestinal tracts. Relating to the lesion to be described, a note was made to the effect that in the left groin there was a pulsating expansile tumor, the size of a hen's egg, situated over the profunda femoral artery. Over this tumor was heard a continuous murmur which possessed a systolic accentuation. The information was obtained that about eighteen years before the patient had been shot through the thigh by a small caliber (probably 22) rifle bullet. The amount of disability at the time of injury was not stated, but it was known that since that time the patient had lived in and had traveled extensively through South America, and had spent much time on horseback.

At autopsy the heart was found to be markedly hypertrophied and dilated. The dilatation was most marked on the right side, the tricuspid orifice admitting five fingers readily. There was considerable atheromatous degeneration in the aortic arch. The bladder, sigmoid, and upper portion of the rectum were found to be displaced forward and to the right by the very tortuous and dilated left common and external iliac arteries and by a fusiform dilatation of the external iliac vein, 6.5 cm. in diameter. The tortuosity of the artery increased its length from the aortic bifurcation to the femoral ring from the average of 15 cm. to about 40 cm. After passing through the femoral ring, where they were constricted, both vessels dilated again to the average diameter of about 2.5 cm. At a point about 6.5 cm. below the origin of the profunda femoral vessels which were of about normal size, the superficial femoral vessels were bound to each other by dense adhesions and communicated with one another through an irregularly rounded smooth edged opening about 0.6 cm. in diameter. On the outer wall of the artery opposite this opening there is a similar opening which leads into a thick walled saccular aneurism. There is a similar opening through the inner side of the vein wall. This opening probably communicated with a sac similar to the one on the artery. Owing to the collapsed state of the vessels, this sac was unfortunately cut through in removing the specimen, but the part of its wall attached to the vein indicates that it was of considerable size. The vein was dilated and its wall thickened for a distance of 3 cm. below the communication, where a pair of well developed and obviously competent valves were found. Below these valves the vein appeared normal in diameter and in the thickness of its wall. Above the communication both artery and vein were about equally dilated up to their point of passage through the femoral ring, where after a moderate constriction they both quickly dilated to form the intrapelvic lesions described above.

The common and the external iliac artery and its continuation as the femoral to the point of communication with the vein showed marked atheromatous change with calcification and ulcerative processes. The corresponding veins with the exception of the large dilatation of the external iliac portion showed marked thickening, which was present also throughout the ascending vena cava, and suggested that the pressure to which it was subjected for many years was also the cause of the cardiac lesions.

Discussion:

DR. NORTHRUP: I have seen recently two cases of artificial communication between the arterial and venous systems. One of these has come to autopsy. The other, a middle aged woman, is now at the Presbyterian Hospital. The most prominent symptoms in this case are pain and cyanosis in the upper part of the body.

REPORT OF A CASE OF FIBROMYOMA OF THE STOMACH.

A. B. EISENBREY, M.D.,

(Resident Pathologist, St. Luke's Hospital.)

The specimen shown was obtained at autopsy on a fifty-three year old female, who entered St. Luke's Hospital in December, 1912, on the service of Dr. Hollis, complaining of swelling of feet, dyspnoea, and weakness, and presenting the symptoms of cardiac insufficiency. She gave no history of any gastric disturbances and none of her symptoms were referable to the lesion in the stomach. Autopsy revealed a dilated and hypertrophied heart and general passive congestion of the organs and atheroma of the large vessels. There were no uterine fibromyomata nor were any other tumors found in the gastrointestinal canal.

The stomach occupied a normal position in relation to the other abdominal organs. Its size was about normal and it contained a moderate amount of dark brown fluid. The serosa presented no abnormalities. The mucosa was somewhat congested and slight post mortem digestion had occurred. In the cardiac end the mucosa was thickly studded with minute petechial hemorrhages. On the external surface of the posterior wall of the stomach, about 4 cm. below the esophageal opening, the area of attachment of the tumor was indicated by a somewhat whiter appearance than that of the surrounding gastric wall. No increase in the size of the superficial vessels was noticeable. The tumor itself projected from the inner surface of the gastric wall. The mucosa covering it appeared thinner, paler, and smoother than that on the surrounding surface. The tumor was firmly attached to the gastric wall. It measured 1.7 x 1.3 cm. and projected 1.1 cm. beyond the surrounding surface. The base was slightly constricted as if pedunculation was beginning. On section the tumor was rather firm and uniform in consistency. The capsule stood out plainly and the substance of the tumor was of a pale, rather fibrous appearance, with evidence of considerable vascularity in the central portion.

Microscopical examination shows an encapsulated tumor made up of interlacing bundles of smooth muscle fibers arising from the inner layer of the muscular coat of the stomach wall. The submucosa and mucosa have been pushed before the developing tumor, and the former in a thinned out and compacted form, acts as the capsule of the growth and, near the point of attachment of the tumor, carries its rather rich blood supply. The mucosa over the surface of the tumor is made up largely of the muscle fibers, and the connective tissue elements are relatively scanty. In general, the structure is rather loose, not very rich in nuclei, and a moderate amount of edema is present throughout. The blood supply is quite rich and consists principally of rather large spaces lined with endothelial cells without definite connective tissue walls. Some of the spaces appear to lack even an endothelial lining. A few wellformed but thin walled vessels are present. The vascularity is most marked in the central portion of the growth where, in a number of areas, the blood has escaped from the larger spaces into the surrounding tissues. Around one of the larger blood spaces near the center of the tumor there is a collection of lymphoid cells with a few plasma cells scattered among them.

A CASE OF GENERALISED MILIARY TUBERCULOSIS WITH NUMEROUS TUBERCLES IN THE SKIN.

DR. W. P. NORTHRUP.

This very unusual case was in the Presbyterian Hospital. It is one of general miliary tuberculosis involving the skin found in a girl two and a half years of age. The child was brought into the hospital with the symptoms and chart of a pneumonia, the physical signs of a moderate bronchitis, and great prostration. A tentative diagnosis of miliary tuberculosis was made and an X-ray picture taken. The picture shows a condition which in jest and with peculiar descriptive accuracy may be named "measles on the lung," an expression which you will see applies to the picture of disseminated tuberculosis without areas of consolidation.

The eruption noticed about the face and neck was suggestive of syphilis. The character of this eruption was that of little sudamina, and as the lesions became older they presented the characteristic appearance of miliary tuberculosis of the skin. Stained smears from the vesicles were pink with tubercle bacilli. The histological features of the lesions indicated that there was no reaction whatever on the part of the body. They were areas of necrosis with no giant cells or epitheloid cells.

At autopsy the lungs were found to be filled with tubercle bacilli, which were likewise present in the liver, kidney and spleen. The blood and urine showed numerous bacilli. Although Dr. MacCallum searched for four hours, opening the bronchi, veins and thoracic duct, he was not able to find the portal of entry. The brain was not opened.

This case is of interest because it is an example of widespread tuberculosis in a child which offered no resistance to the infection. This explains the character of the lesion and the number of organisms present. Beautiful pictures of the eruption may be found in Pfaundler and Schlossmann's book.

The case in full with clinical features will be published elsewhere.

Discussion:

Dr. Lamb: I had an opportunity of observing this case at autopsy. The striking thing about the tubercles is that they are simply areas of necrosis with no sign of fibrous tissue, epitheloid or giant cells. The skin lesions are merely areas of superficial necrosis and swelling of the epithelium, showing large numbers of tubercle bacilli. These are present even in the shedding flakes of the corium.

There were two similar cases in infants at the Presbyterian Hospital four or five years ago. The rash is perfectly characteristic. Tileston examined some thirty-two cases of miliary tuberculosis and found a similar rash in seven of them. In seventy per cent of the cases he found tubercle bacilli. His findings go to show that at least in this type of skin eruption the lesion is not a "tuberculid" caused by the toxin of the organism, but a definite lesion caused by the tubercle bacillus itself.

The rash is generally found about the groin, inner sides of the thighs, and over the buttocks where the skin is kept moist by the diapers. At times, there are only five or six of the lesions present, but Tileston believes that they are sufficient to enable one to make a diagnosis of miliary tuberculosis.

In its characteristic stage the rash consists of little red papules about the size of rose spots in typhoid fever, with a central crust. On removing this crust there is found a very definite little dell or hollow, in the center of the lesion. In Pfaundler and Schlossmann, the lesion is very well illustrated and called "papulo-necrotic tuberculid."

Dr. Ewing: I am extremely interested in this case. I did not learn whether these eruptions were hemorrhagic. My attention was called two years ago to a similar case in which the eruptions were hemorrhagic. This case was presented to the Society at that time. The symptoms illustrate a different condition from the one just described. The patient was an adult woman who had been well until six weeks before death. The symptoms began with a swelling of the lymph nodes of the neck. I made a blood examination on the case and found a relative lymphocytosis and eosinophilia. The eruptions were profuse, hemorrhagic lesions consisting of pin-point petechiae, which were so numerous on the flexor surface of the forearms as to give a red appearance. The patient developed pneumonia and died from it.

At autopsy the lesions were found to be mostly in the lymphatic system. A monkey inoculated from a blood culture died from tuberculosis. Cultures from the lungs and liver were successful on glycerine agar media. On section the lesions were found to consist of areas of local necrosis followed by hemorrhage. They were not miliary tubercles. We have, then, two characteristic types of skin eruption, that described by Dr. Northrup, in which the bacilli are found, and this other case resulting from a toxic condition in which hemorrhagic eruption occurs. This latter condition is sometimes seen in cases of old tuberculosis and serves to draw attention to a recrudescence of the infection.

Dr. Northrup: The lesions found in our case were not hemorrhagic at first but became so as the disease progressed. They began as vesicles, then became hemorrhagic about the circumference and then they dried and became umbilicated. They are best described as sudamina of rather large size.

A CASE OF PERIARTERITIS NODOSA.

ALBERT LAMB, M.D.

The patient who forms the basis of this report was a man, age 26. He entered the Presbyterian Hospital, December 7, 1912, on the service of Dr. Janeway to whom I am indebted for permission to use the clinical records of the case. His past history was negative. He had never had syphilis. The only disease which he remembered was an attack of influenza two years before admission. There was nothing in the family history bearing on the case. Two years before he came to the hospital he began to suffer from asthma at infrequent intervals. More recently these attacks have come on every two or three weeks. Three weeks before admission, a nodular swelling the size of an egg appeared quite suddenly in the region of the right ankle. There was intense itching and the surface of the swelling was covered with small purpuric spots. In the course of a few days this swelling disappeared. From this time until he entered the hospital he suffered alternate attacks of asthma and fresh crops of purpura. The latter affected all parts of his body except the face. Often the eruption was accompanied by severe abdominal cramps and pain or by pains in his various points. During his stay in the hospital, the fresh crops of purpura continued to appear with the abdominal and joint pains. He also had attacks of asthma. He ran an irregular temperature. For a few days it would be elevated to 100°-101° and then there would be a few afebrile days. His pulse corresponded with the temperature but was rather more rapid than the height of the temperature would indicate. His leucocytes varied from 17,000 to 21,000 and the eosinophiles from 20 to 51%. His urine always showed albumin, at times up to 0.1-0.2%. There were always hyaline and granular casts and often epithelial and waxy ones. His phthaline excretion was diminished. The Wassermann reaction was negative. About ten days before his death he had an infected ulcer of his leg and just before the end a positive streptococcus blood culture. During the last two days he had a pericardial friction rub. He died February 6, 1913.

At the autopsy, there was found a fresh streptococcus pericarditis, pleurisy and peritonitis. There were two small vegetations on the mitral valve. No organisms were found in these vegetations.

The lungs showed extreme oedema and were in full inflation. The kidneys were slightly enlarged. The capsule was only slightly adherent. On section the cortex was thin, the markings indistinct and the glomeruli appeared as colorless dots. No nodules of any kind were observed on any of the vessels in the ordinary course of the examination. The diagnosis was not suspected until the microscopical examination was made and then on reviewing the material a few tiny nodules were found on the coronary vessels and on section of the kidneys some of the vessels appeared thickened.

On microscopical examination the changes characteristic of Periarteritis Nodosa were found in the vessels of the kidneys, adrenals, stomach, and heart. The lesion as seen without tracing it through serial sections, showed a marked infiltration of the adventitia and surrounding tissue with mononuclear wandering cells and eosinophiles, necrosis and cellular infiltration of the media with the characteristic hyaline appearing band, and rather marked thickening of the intima. In some places, there was considerable new formed connective tissue about the vessels.

The kidneys showed a marked glomerular nephritis with obliteration of most of the glomeruli.

Sections of the vascular lesions stained for bacteria failed to show any.

A full report of the case will be published later when opportunity has been found to examine the serial sections which are being prepared.

THE PROPERTIES AND AGGLUTINATIONS OF SOME NON-PATHOGENIC VIBRIOS.

C. V. CRASTER, M.D.,

(Quarantine Laboratory, Rosebank, New York.)

During routine examinations for cholera certain monoflagellate organisms morphologically resembling the cholera vibrio are frequently observed. These are usually obtained from persons free from disease, and are not known to cause any lesion in man.

In the routine examination carried out at Quarantine in 1911 over one hundred non cholera vibrios were found. These vibrios with few exceptions closely resembled the cholera organism in morphology and motility. In culture, slight differences were discernible in the colonies and alkaline agar plates. No typical indol reaction was obtained. They were hemolytic to a variable degree, and gelatine was readily liquified. The action on sugars was similar to that of the cholera vibrio. Three of the strains showed pigment formation, and one was a gas former; the latter a character not usually associated with the choleralike group. Pathogenicity tested by feeding experiments and by Pfeiffer's peritoneal inocculation test with living cultures demonstrated no pathogenicity to animals. The non cholera vibrios agglutinated with anti-cholera serum only in very low dilutions (1-10) and not at all in high dilutions. The cholera vibrio is very sensitive to the action of anti-cholera serum, and agglutinated in serum dilutions of 1-10,000.

A series of anti-sera was prepared from twenty varieties of non-cholera vibrios. The various cultures of these were agglutinated freely by the heterologous sera. The positive agglutinations were not, however, always reciprocal. The results of the tests showed the presence of certain group agglutinations and in this way the different non-cholera vibrio cultures could be shown to be related in some degree.

The role of the non-cholera vibrios is still uncertain. They have been regarded as "transitional" forms of the cholera vibrio by some authorities, especially Zlategroff, who has endeavored to prove that the ability to agglutinate with anti-cholera serum may be acquired by subjecting the cholera like vibrios to various cultural and inoculation procedures.

Discussion:

Dr. Krumwiede: A point which seems to me of great importance was brought out in this work, that is, that very few of these non-cholera strains produced indol. In an emergency, when one has no agglutinating serum with which to exclude these vibrios, the indol reaction becomes a very useful one for distinguishing true and non-cholera vibrios. In this connection it might be mentioned that even on direct examination non-cholera vibrios may be very numerous. In the examination of a nurse at Bellevue Hospital who took care of a cholera case and developed diarrhoea, a very large number of vibrios, shown to be of the non-cholera type on subsequent isolation, were found on direct smear from the feces.

Dr. Ewing: · What was the effect of anti-cholera serum on these vibrios?

Dr. Craster: There was some agglutination in low dilutions such as 1-10.

Dr. Wadsworth: In the investigations on the recent epidemic of cholera in Italy it was found (Carapelle. Ann. Sq. Sper., xxii, p. 497-520) that vibrios which failed to agglutinate in cholera serum could be divided into groups, those which agglutinated after passage through the peritoneum of an animal and those which did not agglutinate after this treatment. Has Dr. Craster obtained similar results in any of his studies?

Dr. Craster: I have not had any experience in such tests. Dr. Zlatogoroff found that cholera vibrios could be made to acquire agglutination properties, and his work was confirmed by Barrensheim. McLaughlin and Whitmore repeated Zlatogoroff experiments with non-cholera vibrios isolated in the Philippines but were unable to confirm the conclusions of Zlatogoroff and Berrensheim.

OBSERVATIONS ON THE GROWTH OF BACTERIA ON MEDIA CONTAINING VARIOUS ANILINE DYES.

CHARLES KRUMWIEDE, JR., M.D., AND JOSEPHINE S. PRATT, A.B.

(From the Research Laboratory, Department of Health, New York City.)

In a recent communication Churchman described a selective bactericidal action of gentian violet. Stated in general terms, he found that bacteria were divided into two groups; one group failed to develop when gentian violet was present; the other group, however, grew abundantly on media containing the dye. The former corresponded in general to the Gram-positive group of bacteria, the latter to the Gram-negative group. This difference was most uniform when the organism to be tested was inoculated on agar containing the dye. His conclusions are based mainly on results obtained with dilutions of 1-100,000 of the dye.

A striking variation was noted with some enteritis strains. These strains were identical by all ordinary cultural tests; one strain, however, persistently refused to grow on gentian violet agar. A subsequent examination showed that this strain could be differentiated from the others by agglutination.

Our interest in the use of media containing dye-stuffs was further aroused by an article by Signorelli. He claimed that on agar containing dahlia cholera vibrios developed colored colonies by the absorption of the dye, with decolorization of the medium; whereas non-cholera vibrios did not. We were unable to verify these findings. In the course of the work we found two vibrios which refused to grow in dilutions allowing profuse growth of the other strains. A similar difference was noted by Churchman in certain enteritidis strains.

This observation led us to try a limited number of Gram-negative and Gram-positive organisms on dahlia agar. The results were similar to those obtained with gentian violet. We decided from this that the reaction to gentian violet was a reaction probably common to many allied dyes, and further experiments were begun to decide the question definitely. We used 1-100,000 of the dye in agar. Dahlia showed one exception: our strain of

Shiga failed to grow, whereas it did on gentian violet agar. On rosanaline violet agar the results were the same as on gentian violet agar. Säure violet failed to restrain any of the Gram-positive bacteria. Hoffman's violet was tried, and to our surprise the streptococcus-pneumococcus group grew on the dyestuff agar. On making a solution of another sample of this dye, however, for verification, we detected a source of error in our first experiment. We found by a comparison of the colors that, on account of the slow solution of the dye, the first sample tried had given a dilution of about 1-400,000 instead of 1-100,000, when mixed with the agar. In these experiments two facts were noted: Shiga failed to grow on dahlia, and the streptococcus-pneumococcus group grew on a dilution of Hoffman's violet which still inhibited all the other Gram-positive bacteria used.

The observation on the action of dahlia led us to ask what influence has fuchsin on the growth of bacteria, especially the colon-typhoid-dysentery group. In spite of the common use of Endo's medium there seem to be no observations on this point. We found that fuchsin restrained the staphylococcus and the diphtheria and hay bacillus. Among the typhoid-colon-dysentery group only Shiga was inhibited.

The following table gives the results on fuchsin agar at various dilutions using the available strains of dysentery and para-dysentery. The tabulation gives the range of growth in several experiments in which stock solutions made from two different bottles of dye.

It appears probable from the table that some of the cultures from different institutions are duplicates. They are included because we were unable to determine this point with certainty. Furthermore, they serve as controls on the variability of the reaction.

The reaction falls short of being a specific difference of individual cultures, there being a shading of one group into another. There is, however, a marked tendency to a group reaction. It should be noted here that the seeding was from fresh broth cultures. In this as in the work on cholera we found that heavy seeding cultures obscured the restraining action of the dye and led to irregular results.

Endo being the commonest used medium for the isolation of dysentery it seemed to us almost self evident that the decolorization by sodium sulphite must remove this inhibition or else an unsuitable medium was being used.

An agar containing 1-25,000 decolorized fuchsin was made. The strains showing no growth in the presence of this concentration of the dye after twenty-four hours incubation were planted. Three strains grew as well as on the control, four strains showed slight to moderate restraint and one strain showed marked restraint. With a similar medium containing the dye in 1-100,000 no restraint was evident. Closer observations are necessary to find the danger point in concentration. Evidently decolorization robs the dye of most of its restraining action. We have not made as yet similar tests with other dyes.

The action of gentian violet on these same strains was tested and although the growth of certain strains was remarkedly restrained or completely inhibited the results were not parallel to those obtained with fuchsin. Dahlia, work with which first suggested a variation in the members of the group, has not been tried.

Two strains, one of the Shiga type and the other of the paradysentery type which reacted almost alike, were plated and ten fishings made in broth. These were planted on fuchsin agar to determine the possible range of variation under identical conditions. A moderate variation was noticed, in some instances slightly greater than the variations already given in the table.

Returning to the observation on the streptococcus-pneumococcus group we have tried the growth of this group on both gentian and Hoffman's violet in various dilutions. A large number of other bacteria, Gram-negative and Gram-positive, have also been tried. The results on the two dyes seem to be practically identical, although a complete analysis of the experiments has not been made. The results may be summarized as follows:

In general the Gram-negative bacteria, using representatives of all groups encountered in human and veterinary medicine, were uninfluenced by the presence of the dye. The lowest dilution employed was 1-200,000. Poorly growing bacteria, such as the meningococcus and gonococcus, gave irregular results.

Gram-positive bacteria, leaving out of consideration the pneumococcus-streptococcus group, were all completely restrained with dilutions of 1-500,000, even after forty-eight to seventy-two hours' incubation. Occasionally a limited growth appeared at the beginning of the streak when seeding was very heavy, or where the water of condensation carried the bacteria to the edge of the dyed agar. The latter was more evident when the dilution was increased to 1-600,000.

The streptococcus-pneumococcus group grew freely on agar containing the dye in dilutions of 1-400,000 to 1-500,000. The only exception was a stock culture of streptococcus lacticus. This failed to grow, or showed a feeble growth after prolonged incubation. With a dilution of 1-600,000 of gentian violet it grew well. After a short period of incubation slight restraint was evident in some instances. An increase of the amount of dye led to irregular results. When a dilution of 1-100,000 was reached a few strains were still able to grow after prolonged incubation.

A large number of strains have been tried, including the pneumococcus and streptococcus mucosus types, types from chronic endocarditis, streptococci from various diseased conditions in man, a series of streptococci from the conjunctiva, a series isolated from domestic animals and a few from the external world.

On examining Churchman's protocols in the light of our results it is not surprising to find his greatest irregularities with this group of organisms.

The technic we have employed is evident from an examination of the plates. Agar slants are made in opposite sides of the dish. The method requires little media and is simpler than putting a partition in the plate. We encountered several series of irregularities. When, however, a batch of medium was first tested for its suitability for growing the streptococcus-pneumococcus group, and the plates were used when freshly made and moist, these irregularities disappeared. The use of dye in serum media has not been tested. The control and dye agar were taken from the same batch of medium in every case.

The results described are given mainly for their suggestiveness. Not only should the action of the various dyes be investigated but their quantitative influence should be determined. The possibility of finding further biological differences, and their application to selective cultural media does not seem remote.

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*We are indebted to the Museum of Natural History, the Rockefeller Institute, and to Drs. Hiss, Torrey and Williams and Miss Broadhurst for many of the cultures used in this work.

Note.—A paper, entitled "The Action of Pneumococcus on Blood," by Dr. E. E. Butterfield and Dr. F. W. Peabody, was read at the December meeting of the Society. It will be published elsewhere.

GROWTH OF DYSENTERY BACILLI ON FUCHSIN AGAR

Culture	Mannite Fermentation	Time of Incubation and Dilution of Dye										
		18–24 hours				48 hours				72 hours		
		25,000	50,000	100,000	150,000	25,000	50,000	100,000	150,000	25,000	50,000	100,000
Shiga — Gay-Shiga — Shiga	—	—*	—	—	— or §		—	s.c. or §	‡	—	s.c.	‡
Kruse — Kruse	—	—	—	— or s.c.	— or §	—	—	s.c. or §	‡	—	s.c. or §	‡
Kruse — Shiga	—	—	—	— or §	§	—	—	§ or ‡	‡	—	§	+
Shiga	—	—	—	— or §	‡	—	— or s.c.	§ or ‡	‡	—	‡	+
Torrey, 21	—	—	—	§ or ‡	‡	—	§ or ‡	+	+	— or 1 c.	§ or ‡	+
Cruz, 1	—	—	— or §	‡ or +	+	— or §	‡	+	+	— or §	‡	+
Sydenham	+	—	— or §	‡ or +	+	—	§ or ‡	+	+	—	‡	+
Torrey 4, Torrey 5 6, Hiss — Y	+	—	§	‡ or +	+	s.c. or §	‡ or +	+	+	§ or ‡	‡	+
Hiss-Y, Flexner-Harris, Medburg, Ferra-Hiss, Torrey 16-3, Torrey 32, Y, Baltimore, Strong, Flexner, Mt. Dessert	+	§	‡ or +	‡ or +	+	§ or ‡	‡ or +	+	+	‡	‡ or +	+
Strong, Gay-Flexner, Silk, Balt.—Flex., Gray, Torrey 3, Torrey 41 Flexner-Harris	+	‡	‡ or +	+	+	‡ or +	+	+	+	‡ or +	+	+
Gray, B., 48 Hiss	+	+	+	+	+	+	+	+	+	+	+	+

* Growth: —, no growth; *1 c.*, one colony; *s.c.*, several colonies; §, very slight growth; ‡, evident restraint; +, practically like control

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DR. A. B. WADSWORTH, *President*

DECIDUAL REACTION IN THE APPENDIX

ELI MOSCHCOWITZ

The appendix was removed in the course of an operation for tubal pregnancy. The right tube was occupied by a blood clot about the size of a walnut. Pregnancy was demonstrated by the presence of typical chorionic villi.

The appendix was two inches long, uniform in thickness and the diameter of a lead pencil. The peritoneal surface was glis-

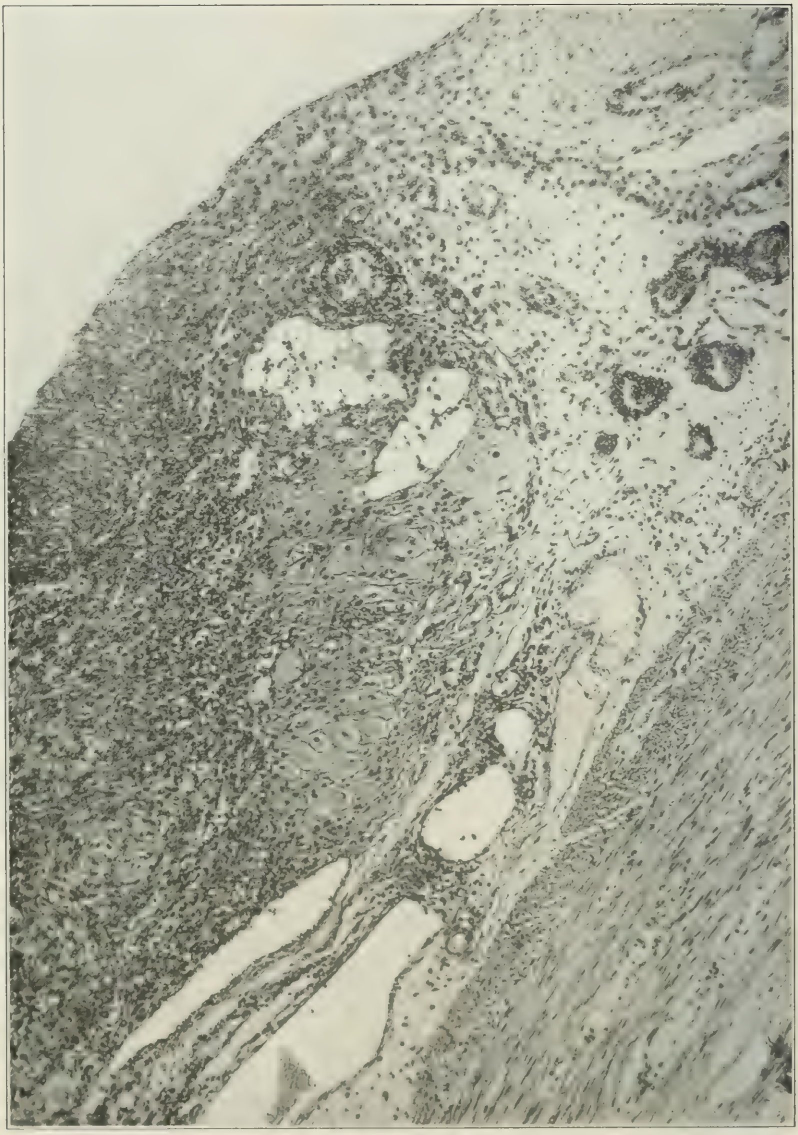

FIG. I.

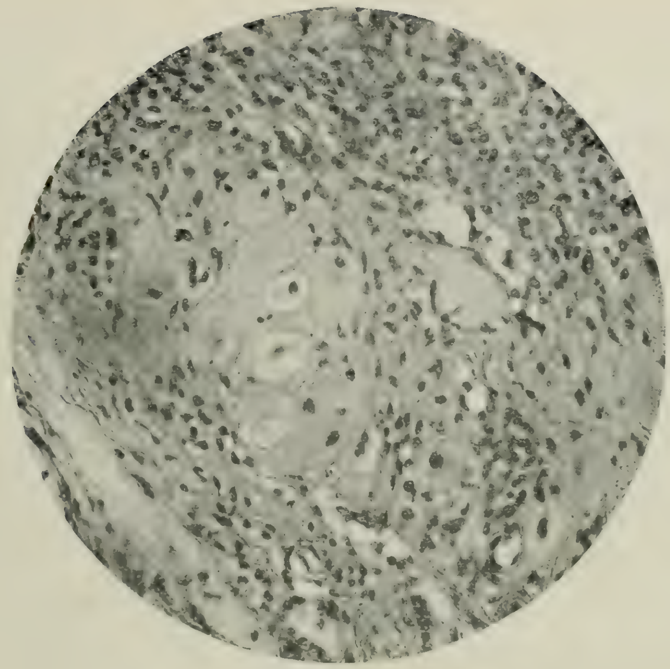

FIG. 2.

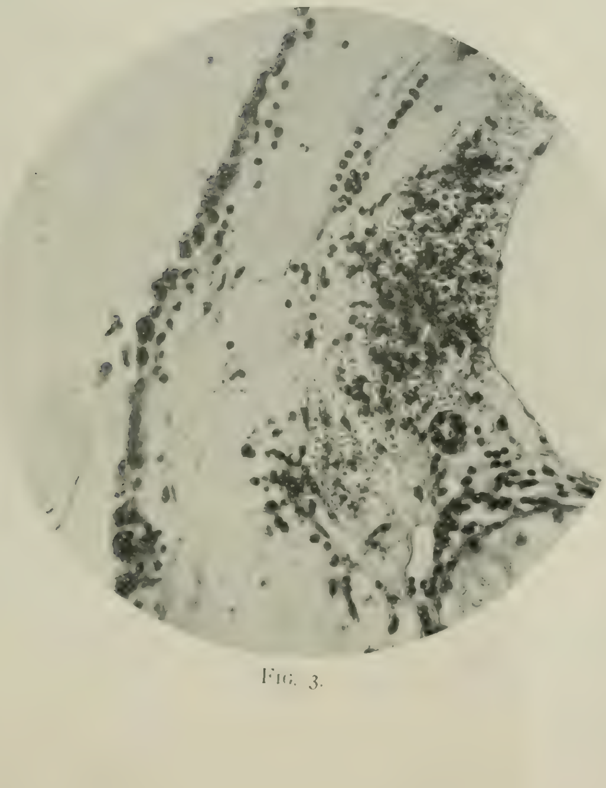

FIG. 3.

tening, white, and there were no adhesions. Grossly, in other words, the appendix was normal. The organ was situated in its normal position in the right iliac fossa and, according to the surgeon, was at a considerable distance from the right adnexa.

Microscopically, the appendix was entirely normal except for the subperitoneal connective tissue. Around the entire circumference, but most prominent at the junction of the mesenteriolum, the subperitoneal connective tissue contains many large round, ovoid or elliptical cells. These cells lie discretely, the cell bodies are pale and finely granular. The nuclei are small, round, eccentric and stain rather deeply. The cells resemble in every way the characteristic decidual cell found in the genital tract in normal pregnancy (fig. 1).

Between these decidual cells are large numbers of ordinary round cells which are more abundant, especially at the site of the mesenteriolum, than in the normal appendix (fig. 2).

The peritoneal endothelial cells are prominent and are decidely more swollen than in the average normal appendix (fig. 3).

The writer has examined about half a dozen appendices removed in cases of tubal pregnancy but this is the only instance in which a decidual reaction was found. In two cases the appendix was even adherent to the ectopic sac.

This decidual reaction in the appendix has been noted once before: Hirschberg, *Archiv für Gynekologie,* LXXIV, page 620.

MIXED TUMOR IN A PHEASANT

C. V. CRASTER

Tumors in birds, and more especially those in the domestic fowl, have been frequently described in recent literature. In the earlier records are those of L. Pick (1), who drew attention to the occurrence of a squamous-celled carcinoma in the mouth of a hen. A similar neoplasm was described at a later date by M. Koch (2). In 1902, Ehrenreich (3) made an extensive examination of the bodies of 4,000 fowls killed for food, and stated that neoplasms were of comparatively frequent occurrence. He found seven malignant growths, five of which were carcinomata of a glandular type, in one the epithelium was grouped in solid masses. There was one sarcoma, made up of round cells, with large nuclei, with small amount of cytoplasm, and two fibromata.

Tyzzer and Ordway (4) record the finding of nine tumors in fowls. One of these was a myxosarcoma of the thigh, which on section appeared to be of almost a gelatinous consistence, with central necrotic areas. In this tumor malignant infiltration of the adjoining tissues was observed. Some of the cells were stellate in form. The other tumors were six lymphomas, in various parts, including the abdomen and neck, with infiltration and metastases, and one leiomyoma of the mesentery. More recently the spindle-celled sarcoma of the hen, of Rous (5), the myxosarcoma of Fujinami and Inamato (6), and the osteochondrosarcoma of Tytler (7) are important by reason of their successful propagation by serial inoculations into other fowls.

The tumor here described was found in a Reeve's pheasant (*Syrmaticus Reevesi*). The autopsy showed a soft mass occupying the greater part of the abdominal cavity; the intestines were compressed backwards, where they occupied a small space against the vertebral column; the liver and diaphragm were considerably displaced upwards, the heart occupying a high position in the thoracic cavity.

Apart from the tumor there did not appear to be any other pathological condition present. There were no metastases and no infiltration of adjacent tissues; the organs were normal, and there was a deposit of fat around the abdominal and thoracic viscera. Cause of death was obscure; there were no signs of any concurrent acute infection.

The neoplasm was found to be lying free in the abdominal cavity, with the exception of a very slender pedicle connecting it with the posterior abdominal wall at the junction of the lumbar

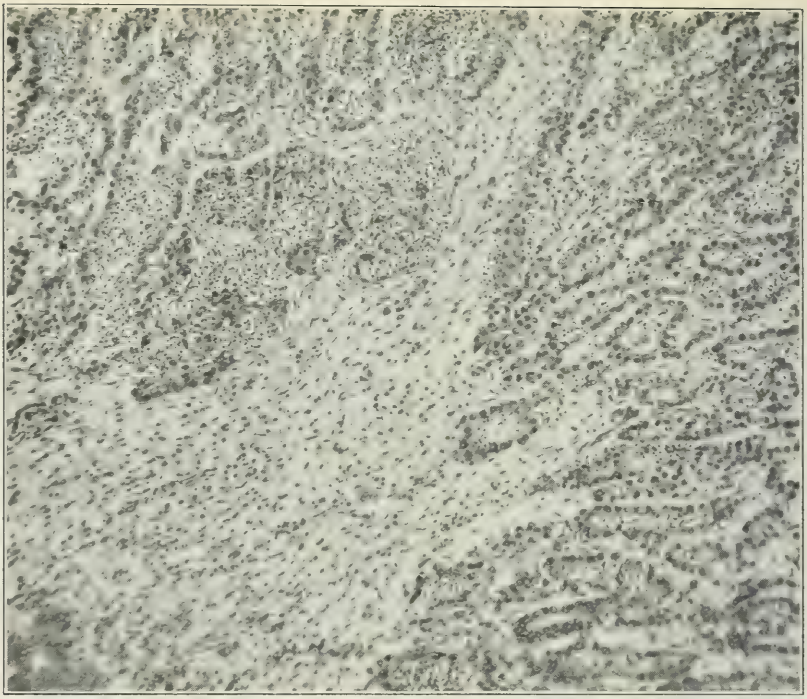

FIG. 1.

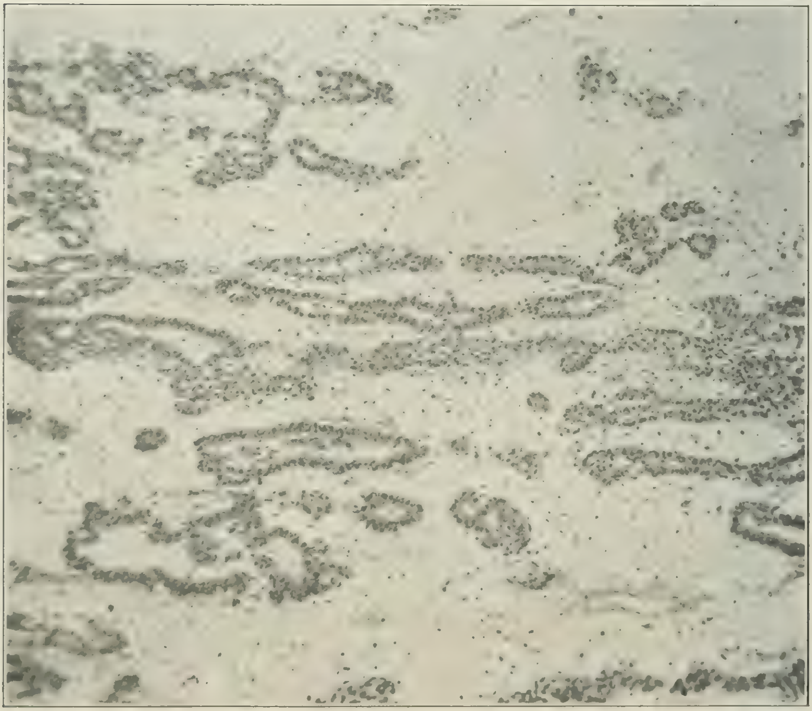

FIG. 2.

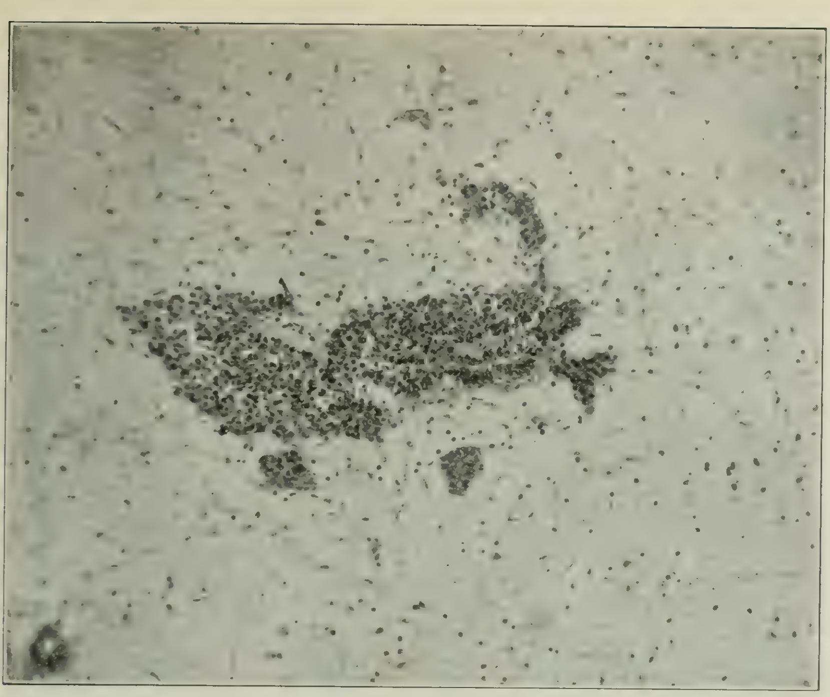

FIG. 3.

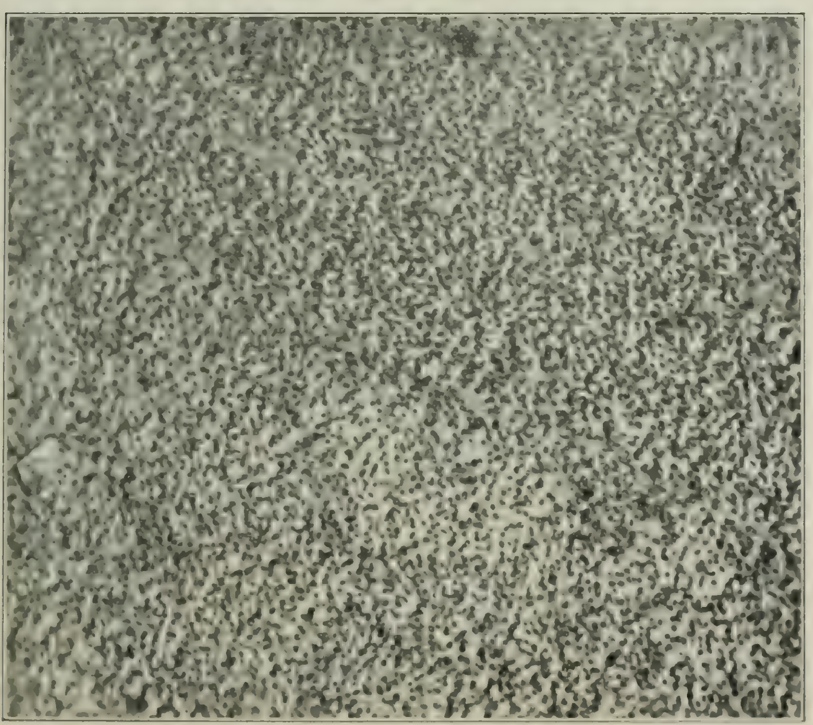

FIG. 4.

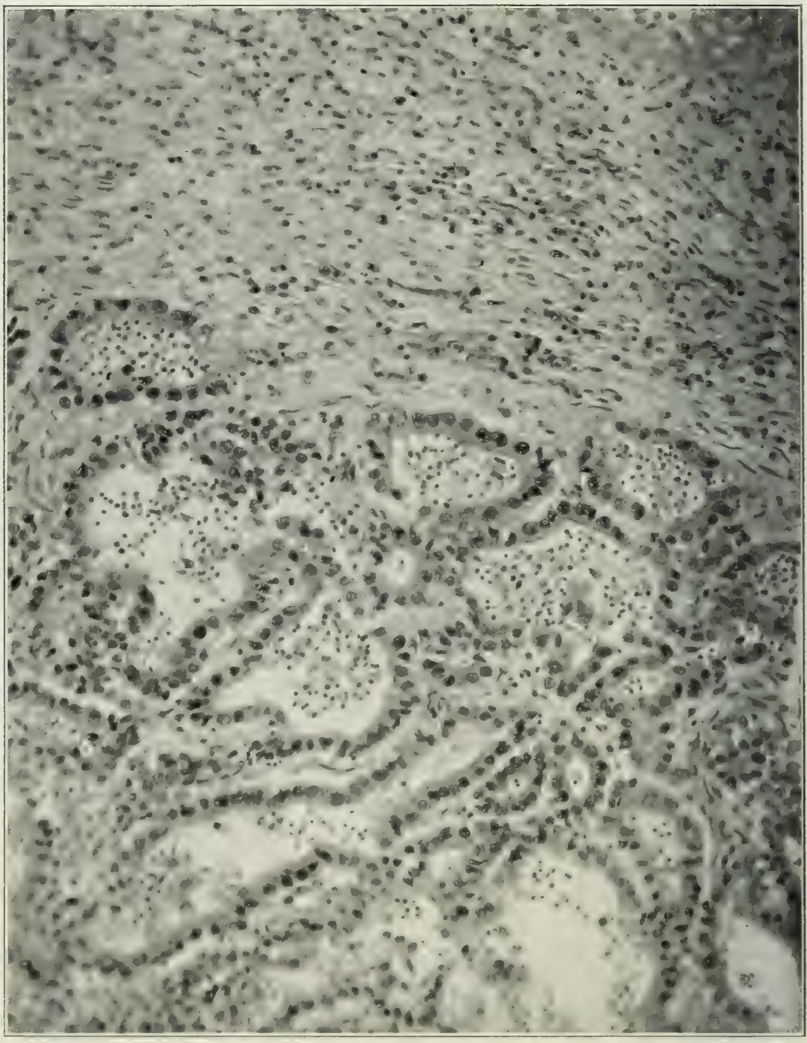

FIG. 5.

and sacral vertebræ. The weight of the tumor was 160 grams. On superficial examination it appeared as a soft dark yellowish lobulated sarcoma-like growth, surrounded by a loose fibrous capsule, which was thinned to a transparent membrane over surface cavities, through which the color of the contents was plainly visible, giving the whole tumor a mottled appearance.

On section the tumor substance appeared to be made up of a soft fibrous material in which cyst-like cavities were found, some large, containing blood, necrotic and mucoid material. The cavities were separated from each other by fibrous partitions of a denser nature, varying in thickness from one to two millimeters.

MICROSCOPICAL APPEARANCES

The microscopical appearance showed the presence of tissue elements, of various types. These may be said to be demarcated into areas of four types:

(1) Adenomatous tissue with fibrous interstitial substance (fig. 1).

(2) Adenomatous tissue with myxomatous interstitial substance (fig. 2 and fig. 3).

(3) Small round-celled sarcoma-like tissue (fig. 4).

(4) Spaces lined with a single row of cuboidal epithelial cells (fig. 5).

(1) *Adenomatous tissue with fibrous interstitial substance.*—A large part of the tumor substance on microscopical examination is seen to be composed of masses of cuboidal epithelial cells, arranged in the form of processes, resembling somewhat those in the canalicular form of the fibro-adenomata of the breast. The cells are in the form of lining membranes, one layer thick, except in some parts of the tumor where the adenomatous-like arrangement is lost, the glandular type of cells being massed together with no definite arrangement. The interstitial substance is fibrous tissue, which sends prolongations of varied thickness into the tumor substance, enclosing and ramifying amongst the glandular cell masses. The fibrous tissue does not enter the adenomatous branches to form an internal supporting framework as

in the papillomata. The shape of the glandular cells is regularly cuboidal. The cytoplasm is slightly opaque, and sometimes contains fat droplets. The nuclei are oval, clear, large, deeply staining with hematoxylin, and there is well marked chromatin network. Mitoses are not evident.

(2) *Adenomatous tissue with myxomatous interstitial substance.*—The glandular cell processes are seen in some portions of the tumor to have a ground substance made up of stellate and irregularly shaped connective tissue cells, in a clear transparent matrix having a myxomatous, or, in some parts, a gliomatous appearance (fig. 2 and fig. 3). In these parts necrotic patches staining faint pink with eosine are frequent.

(3) *Small round celled sarcoma-like tissue.*—Particularly noticeable are large areas of the tumor substance composed entirely of very small round sarcoma-like cells with large dark staining nuclei, and little cytoplasm (fig. 4). There is little or no intercellular substance, and necrotic areas are irregularly dispersed through it. The adenomatous cell masses do not invade this part of the tumor substance.

(4) *Spaces lined with cuboidal epithelial cells.*—The adenomatous tissue forms in places spaces or channels, lined by a single layer of cuboidal cells, more or less completely filled with nucleated erythrocytes and round cells having the appearance of leucocytes (fig. 5).

The cuboidal shape of the glandular cells is constant throughout the tumor substance, and the size does not vary. In some of these cells there is evidence of fatty infiltration. In parts of the tumor where the adenomatous arrangement of the glandular cells is lost, the ground substance may be made up of the very small sarcoma-like cells, or nucleated blood cells.

CONCLUSIONS

The histological structure of this growth in some respects resembles that of the mixed tumors of the parotid gland, since we have epithelial gland tissue, mucoid interstitial substance and various other blastodermic elements. A similarity to the renal teratoblastomas is suggested by the soft sarcoma-like consistence, with the tendency to hemorrhages and necrosis, and by the presence of areas of sarcoma-like tissue.

The cylindrical arrangement of the epithelial processes and the channels enclosed by a single layer of cuboidal cells give a certain resemblance to the canalicular form of the fibro-adenomata of the breast. The spaces and channels filled with nucleated blood cells suggest the possibility that we may be dealing with one of the transitional lepidomata, originating from the endothelium of blood vessels, the so-called hemangio-endothelioma.

Apart from the histological structure, the tumor was of interest as having occupied nearly all the abdominal cavity of the host; its probable non-malignant nature is suggested by its slight pedunculated attachment to the posterior abdominal wall.

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Discussion:

Dr. MacCallum: I saw this specimen in the fresh state and have also seen the sections. It is impossible to decide definitely as to the character of the tumor but it seems safest to classify it as a teratoma, especially since from its pedunculated manner of growth it is obviously benign. There are masses of cells which might be regarded as either endothelial or epithelial. I think a diagnosis of endothelioma is hardly justifiable unless one can trace definitely the origin of the cells from endothelium.

CILIATED CYSTS OF THE ŒSOPHAGUS

ALWIN M. PAPPENHEIMER, M.D.

The first cyst of the œsophagus described in this report was an unsuspected finding at the autopsy of a female infant aged 8 weeks, which showed no other lesions of note. Since it had been observed that the baby took its feedings with difficulty, it is probable that the cyst, which caused a distinct narrowing of the œsophageal lumen, was at least in part responsible for the infant's death.

The cyst measured 18 x 11 x 7 mm. and was situated 4.5 cm. above the cardia on the posterior wall of the œsophagus. The overlying epithelium was thinned out and translucent. The cyst contents were thin and colorless. No communication existed either with the trachea or œsophageal lumen, into which the cyst bulged.

Microscopically, the cyst is lined with epithelium which on closer study presents some interesting features. It is from two to four or five cells deep. The lowermost cells rest upon an indistinct basal membrane, and are characterized by the possession of small, slightly irregular, deeply staining nuclei. The cells superficial to this layer are of several types. One may distinguish cells with a cuticular border and very distinct long cilia. The greater portion of the cyst cavity is lined with cells of this type. Scattered amongst these, and occasionally opening into the cavity of the cyst, are typical goblet cells, the nucleus of which is compressed to a crescent lying at the base of the cell. In addition, there are a few larger rounded cells with clear cytoplasm and oval vesicular nucleus. Some of these are ciliated, others not; in either case they are readily distinguished because of their large size and clear protoplasm, from adjoining cells.

The epithelial lining of the cyst rests upon a bed of loose fibrillar connective tissue, about which are well-developed bands of smooth muscle fibers. In one section, there is found amongst these, a cross section of a tubular gland lined with a double row of non-ciliated epithelium. In the absence of serial sections, it was not determined whether this communicated with the cyst cavity.

No other structures were found in the sections examined. The absence of plates of cartilage, mucous glands, lymphoid follicles and striated muscle fibers should be emphasized.

The œsophageal epithelium overlying the cyst is somewhat thinned as compared with that at a distance, but is not otherwise abnormal.

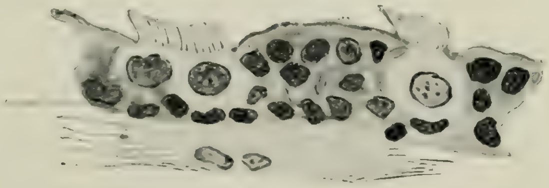

FIG. 1.

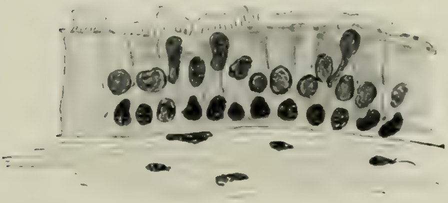

FIG. 2.

In the second case, the cysts were microscopic, and were discovered by Prof. MacCallum in a section of œsophagus taken from a small area, the gross appearance of which is described as follows: "in the upper part of the œsophagus, at the level of the larynx, there is a small ulcerated area about 5 mm. in diameter with slightly raised pearly white borders. Otherwise the œsophagus is normal."

The tissue was taken from a male, 24 years of age, who died of cardiac valvular disease.

The section shows a small plaque of mucous membrane of a character wholly different from that of the adjacent squamous epithelium. It resembles gastric or duodenal mucosa, being composed of high cylindrical epithelium lining irregular tubules or crypts which extend down into the submucosa. The latter is densely infiltrated with lymphoid cells which in two places are aggregated in the form of follicles. The surface epithelium is largely desquamated. At the margin of the plaque, there is a nest of mucous glands, resembling Brunner's glands in the duodenum. The adjoining stratified epithelium at the edge of the plaque stops abruptly and there is no transition. It is twice as thick as the normal squamous epithelium at a distance from the plaque, and is penetrated by wandering lymphoid cells.

Three small cysts are found in the sections. They lie not beneath the patch of mucous membrane but to either side of it in the connective tissue of the œsophageal wall. They are of relatively simple structure. The somewhat irregular cavities are lined with a double row of epithelium, the inner cells of which are in most places distinctly ciliated. The basal row of cells contains darker, more spherical nuclei. There is no distinct basement membrane. The cysts are surrounded by a loose connective tissue in which are many lymphoid cells. They are scattered and not in the form of follicles. There is no proper muscular coat to the cysts, as in the first case.

Reported cases of ciliated cysts situated in or adjacent to the œsophagus are not numerous. I have been able to find reference to but fourteen cases, a brief summary of which follows:

v. WYSS: *Virch. Arch.*, 1870, li, 143.

Adult. Cyst, size of an apple, situated 1½ inches above the cardia on the posterior wall of the œsophagus. The cyst was filled with cloudy fluid. The epithelial lining was lost. No communication with the œsophagus.

ZAHN: *Virch. Arch.*, 1896, cxliii, 179.

Adult. Cyst 26 x 18 mm. lying in the posterior œsophageal wall at junction of œsophagus and stomach. The cyst was lined with ciliated epithelium which was in part stratified. There was a well-developed muscular coat, in which were canals and glands. There was also a lymphoid infiltration, the cells in places being massed in the form of follicles. The derivation of the cyst is attributed by Zahn to a dislocation of œsophageal epithelium during early fetal life.

RAU, F.: *Virch. Arch.*, 1898, cliii.

Cyst, 4 cm. in diameter, located at the transition of cardia and œsophagus, on the right posterior wall. The cyst was lined with ciliated epithelium of relatively simple structure.

Trespe: *Arb. aus d. path.-anat. Abtteil. des hygien. Inst. zu Posen von Lubarsch,* Wiesbaden, 1901.

Male infant, less than two months of age. Bilocular cyst, size of pigeon's egg, situated between thoracic aorta and œsophagus 4 cm. above the cardia. The cyst did not communicate with the lumen of the œsophagus, but lay partly within the œsophageal musculature, on the posterior wall. Histologically, the cyst was lined with ciliated epithelium, but contained in its wall scattered plates or "rings" of hyaline cartilage.

From the resemblance of the cyst wall to trachea or bronchus Trespe concludes that the cyst arose through a dislocation (Abschnürrung) of tissue derived from the fetal fore-gut, before or during its differentiation into respiratory and œsophageal portions.

Kraus: *Nothnagel's Hdbch. d. Spez. Path., etc., Erkrankungen d. Mundhohle u. d. Speiserohre,* 1902.

Male, 25 years of age. Cyst in anterior wall of œsophagus, at level of tracheal bifurcation, 6 x 3 cm. in size. The cyst was filled with brownish fluid and lined with ciliated epithelium.

v. Skopnik: *Inaugural Diss.,* Munich, 1907.

The cyst was found in a two-year old boy, dying of diphtheria. It measured 13 mm. in diameter, and was situated in the lower part of the œsophagus, towards the right and anteriorly. It contained cloudy, viscid fluid, and was lined with rather low ciliated epithelium.

Durck: *Muench. Med. Woch.,* 1907, liv, 2165.

Male child of 2 years dying of diphtheria. In the lower part of the œsophagus to the right of the median line, there was found a cyst the size of a hazel nut, with translucent walls. Sections showed a continuous lining of cylindrical ciliated epithelium surrounded by the double layer of smooth muscle fibers.

(Probably the same case as v. Skopnik.)

Mohr: *Ziegler's Beiträge,* 1909, xlv, 325.

Case I.—Woman, aged 81 years. Two-chambered cyst, each cavity about the size of a walnut. The lower pole of the mass, which bulged into the pleural cavities, lay about 3 cm. above the cardia. The cyst was lined for the most part by a single layer of high cylindrical ciliated cells, beneath which there was a row of somewhat more irregularly disposed globular cells with pale protoplasm. In one place, there was found in the wall of the cyst, a plate of cartilage, surrounded by connective tissue.

Case II.—Male, 28 years old, dying of pulmonary tuberculosis. Walnut-sized cyst, 5 cm. above the cardia, on the left side. The cyst was lined with ciliated epithelium, thrown up into papillary folds. Outside of the connective tissue tunica propria which was in places infiltrated with lymphoid cells, were bundles of smooth muscle fibers.

Case III.—Male, aged 65 years, dying of arterio-sclerosis and encephalomalacia. One cm. above the trachea, on the left and posterior wall of the œsophagus, a cyst measuring 3 x 2 cm., bulging into the lumen of the œsophagus, and covered by smooth mucous membrane. The cyst was lined by a simple layer of cylindrical ciliated epithelium, thrown up in places into papillæ.

Staehelin-Burckhardt: *Arch. f. Verdauungskr.,* 1909, xv, 584.

Female, 9 months old, operated for spina bifida. Cyst measuring 5.8 x 4.5 x 2.5 cm. just above diaphragm. The surface covered by pleura save where the cyst was in contact with the œsophagus. The epithelial lining was of variable thickness, in places consisting of a row of simple cuboidal cells, in other portions ciliated, in still other regions presenting definitely the character of gastric mucosa, with glandular crypts. In the sub-mucous connective tissue, follicle-like aggregations of lymphoid cells. There was a distinct muscularis mucosæ, and muscular layer.

The origin of the cyst is attributed to an early misplacement of œsophageal epithelium, including the anlage of cardiac glands. The latter, in their further differentiation, gave rise to the gastric mucosa.

Kern: *Virch. Arch.,* 1910, cci, 135.

Male child of 7 months. On dissecting œsophagus from trachea, a pea-sized cyst was discovered, lying in the tunica adventitia of the œsophagus at the level of the upper pole of the thyroid. The cyst was divided into three chambers, separated from each other by connective tissue septa. The cyst was lined by a single layer of ciliated cells. The wall was composed of connective tissue in which were found plates of hyaline cartilage, mucous glands and ducts lined with cuboidal epithelium. Smooth muscle fibers were not found.

Because of the presence of cartilage and mucous glands, Kern interprets the cyst as an evagination and subsequent constriction of tissue derived from the trachea, and not from the œsophagus.

Stoeber: *Ziegler's Beiträge,* 1912, lii, 512.

Infant of 3 months, upon which had been performed a tracheotomy and subsequent thymectomy for supposed respiratory obstruction. At the autopsy, there was found a pigeon-egg sized tumor on the anterior and lateral wall of the œsophagus, extending from about 1 cm. below the lower pole of the thyroid to within 2 cm. of the bifurcation of the trachea. The cyst measured 5 x 3 cm. It lay directly beneath the mucosa of the œsophagus, and did not communicate with the lumen of the œsophagus or trachea. On section, it was unilocular.

Microscopically, the cyst was lined by two to five layers of epithelial cells, in part ciliated. There were occasionally ducts lined wtih cubical epithelium in the cyst wall. Striated muscle fibers were found in places amongst the smooth muscle bundles of the cyst wall. On closer study of the lining epithelium of the cyst, various types of cells were found corresponding to those described by Schridde as occurring in the fetal œsophagus. Amongst these, was a single group of very high cylindrical cells, probably representing an island of gastric mucosa.

Coesfeld: *Inaug. Diss.,* Kiel, 1891 (not accessible).

From the cases described, it will be seen that there is considerable variation in the size of the cysts. The largest is that reported by Kraus which measured 6 cm. in length. The cysts vary further, in their location, several having been situated at the cardia, others at various points as high up as the thyroid cartilage. The sites of predilection seem to be either at the junction of œsophagus and cardia, or in the neighborhood of the tracheal bifurcation. They may be found either in the anterior or the posterior wall of the œsophagus. In none of the cases was there a communication with the lumen of the gut.

As regards the finer structure of the cysts, there appears to be considerable uniformity in the character of the lining epithelium. This is always, at least in part, ciliated; it may be either in a single layer, or more frequently, there are two or more layers of cells. In addition to the ciliated cells, cells of various types such as occur in the normal œsophagus during fetal life, may be found. In the article of Stoeber will be found a detailed and well-illustrated study of the different types of cells found in the lining epithelium. With the exception of high cylindrical gland cells of the gastric type, all the forms depicted by Stoeber were found in the writer's case.

The epithelium rests upon a loose connective tissue in which collections of lymphoid cells may be found. A definite basement membrane is not noted in any of the cases. The cysts are almost always surrounded by bands of smooth muscle fibers; only in Stoeber's case is there mention of striated fibers.

Plates of cartilage are described in the cases of Mohr, Trespe and Kern. Whereas the last of these writers for this reason believes the cysts to be derived directly from the trachea, Trespe and Mohr are inclined to believe that the presence of these tracheal constituents indicates merely a very early separation of the tissue from the œsophagus, before or during the time of the earliest development of the respiratory bud. Bert and Fischer have gone a step further, and interpret all ciliated œsophageal cysts, whether or not containing cartilage and mucous glands in their wall, as accessory lung anlages. They thus consider them comparable to the accessory lungs found in the pleural cavities, and occasionally below the diaphragm. They maintain that the ciliated epithelium and the plates of cartilage may be taken as indicating the tendency of œsophageal tissue, when separated from its connections, to undergo differentiation along the line of development of the respiratory system. Stoeber, on the other hand, sees in the ciliated character of the epithelium merely a persistence of the embryonic type of œsophageal epithelium, and the other types of cells as comparable also to the varied forms into which the basal layer is differentiated during fetal life.

It is quite possible that both views are correct; that in the simpler cysts, we are dealing merely with a misplacement of fetal œsophageal tissue, whereas in those cysts the structure of which is complicated by the presence of cartilage and mucous glands, we have in addition to a displacement of œsophageal tissue, a tendency toward the development of a rudimentary lung anlage. The fact that these cartilage-containing cysts are occasionally given off from the posterior-wall of the œsophagus, does not negative this interpretation, since we are dealing with aberrant, anomalous structures.

REFERENCES:

Schridde, H.: Die Entwickelungsgeschichte des menschlichen Speiserohrenepitheles und ihre Bedeutung fur die Metaplasielehre, Wiesbaden, 1907.
Bert u. Fischer: Uber Nebenlungen und versprengte Lungenkeime. *Frankf. Ztschr. f. Path.*, 1911, vi, 27.

SECONDARY CARCINOMA OF THE LIVER

D. S. D. JESSUP

The gross specimen of the liver is presented as of interest on account of the extensive metastases which it shows. It was removed from a woman of 51 years in whom autopsy showed lobular pneumonia and marked jaundice. The primary new growth was in the left breast and was a small nodule less than 2 cm. in diameter showing a scirrhous carcinoma accompanying an adenoma. There had been a history of a lump in this breast dating back some 15 years. The only other metastases were in the axillary, the bronchial and mesenteric nodes near the head of the pancreas.

The liver weighed 4,420 grams; was dark green and showed on both surfaces many yellowish brown areas 1.5–2.5 cm. in diameter. The larger ones are depressed at the center giving an umbilicated appearance. They extend downwards 1 to 2 cm. and similar nodules are scattered all through the organ.

Microscopically the growth is similar to that in the breast. There is much dense connective tissue, and necrosis of areas of tumor cells. The liver cells adjacent to the nodules are compressed and flattened.

ADRENAL REST IN THE OVARY

D. S. D. JESSUP

(From the Pathological Department of the Sloane Hospital)

The specimen presented was obtained from a newborn child at the Sloane Hospital for Women. Death occurred on the sixteenth day and autopsy showed a lobar pneumonia and empyema.

Section of one of the ovaries shows a circular group of cells lying in the cortex which from their individual structure and grouping in cords gives the picture of adrenal tissue. The cells in the periphery have a compact round nucleus and a smaller darker stained cell body than the cells of the central portion. They lie in rows or cords, one to three cells deep, which lie parallel to the circumference. Compared with the suprarenal of a newborn child they resemble the cells of the zona glomerulosa while the cells of the central portion are like those of the zona fasciculata. The cords of the latter run in various directions. There is a rich network of capillaries lying between the cords.

Accessory adrenals are very often found near the gland itself.

Marchand, in 1883, was the first to describe them in the broad ligaments and they are therefore often spoken of as Marchand's adrenals. Different writers have noticed their presence in males in the rete testis, between the rete testis and the epididymis, in the paradidymis and along the cord, and in females, in the ovaries, on the tube, and in the broad ligament. Adrenal tissue has also been found along the spermatic and ovarian veins and in the retroperitoneal tissue below the lower pole of the kidney. Broman (*Normalle und abnormale Entwicklung des Menschen*) thinks that most of the cases described as adrenal remnants in the ovaries are where cells of shrunken corpora lutea are taken for adrenal cells and Schickele has shown that so-called adrenal nodules on the tubes are merely collections of peritoneal epithelium.

In newborn children they have been described by Rieländer as occurring in the broad ligament in 3 out of 16 cases examined.

These adrenal "rests" may go on to the formation of tumors in the ovary and are therefore of interest in certain types of ovarian new growths. Such tumors have been described by Peham and Von Willer.

Discussion:

Dr. Frank: I think that one might discuss Dr. Jessup's specimen and the cyst of the œsophagus reported by Dr. Pappenheimer together. They both occurred in new-born children. It appears to me that very little systematic attention has been paid to the fetal "rests" in different organs. The only man who has classified and reported a large number of cases is Robert Meyer. He has been paying strict attention to these evidences of fetal "rests" for a number of years. His last article reported some 400 cases of different fetal "rests" which he had found in different portions of the body, most of them in the female genital tract, and some œsophageal "rests." He has had so many specimens that he has been able to make a very good attempt at definite classification.

An adrenal "rest" in the ovary apparently is a very rare condition. I personally have never seen one. In examining the specimen I do not think that there is any question as to its interpretation. It might afford an explanation for certain tumors seen in the adult which are usually classified as teratomas. The cells found in these tumors, classified as carcinoma and sarcoma according to the distribution of the stroma, certainly are strikingly like those in this adrenal "rest," and while the tumors in the adult do not correspond to hypernephroma, certain portions of these tumors regularly bear a certain resemblance to them. The cystic œsophageal "rest" bears out what Robert Meyer has said in connection with the 10 or 12 specimens which showed displacement or fetal irregularities in formations in the œsophagus. In most instances he found cardiac glands included in the displacement. While these finds are exceptional they are of extreme importance in relation to tumor formation in the various organs.

Dr. MacCallum: I have been struck by the very marked umbilications in the nodules in the liver in Dr. Jessup's case of secondary carcinoma. The explanation of the umbilication is evident upon making sections of one of these nodules, for it is found that many of the tumor cells in the central part are necrotic while the stroma remains in that situation and is able to contract and pull down the surrounding tissue into a dell.

THE WASSERMANN REACTION IN CANCER

FREDERICK J. FOX

Dr. Fox reported the results of 212 Wassermann reactions done on patients suffering from cancer. Only two cases in the series gave a positive reaction, one giving a + + +, the other being weakly positive. Cases from which a history of lues could be obtained were excluded from the series.

The following table shows 210 cases of various kinds of tumors, all of which gave negative results.

Epithelioma of	
Lip	16
Cheek	7
Chin	1
Scalp	2
Ear	5
Nose	6
Back	1
Penis	2
Eye	5
Hand	1
Vagina	3
	49

Carcinoma of	
Breast	59
Uterus	12
Abdominal viscera	12
Stomach	1
Glands of neck	31
Tongue	21
Rectum	5
	141

Sarcoma of	
Face	3
Breast	1
Scalp	1
Neck	2
Back	1
Thigh	3
Testes	2
Mastoid	1
Eye	1
Sarcomatosis	2
	17

Glioma of brain	1
Malignant papilloma of tongue	1
Papilloma of chest	1
	3

The two following cases gave positive reactions: (1) An unmarried girl, 21 years of age, a domestic. At 12 years of age a "corn" appeared on her right heel. She cut it off several times but it always returned. At 18 years of age she had x-ray treatment, and during the following two years, two operations. Two months before coming to the Skin and Cancer Hospital a second tumor appeared on the inner side of the right leg, and shortly afterwards a third appeared in the right groin. On admission to the hospital the Wassermann reaction was strongly positive in the blood serum. One month later it was found to be still positive and an intravenous injection of salvarsan was given with apparently no result. A section was then removed from the original tumor and a diagnosis of sarcoma made. Six weeks after the administration of the salvarsan the Wassermann was negative.

(2) The second case was that of a sailor 58 years of age, with a very extensive recurrent cancer of the tongue and cervical glands. The Wassermann reaction ten weeks after operation was weakly positive. The patient at this time was anemic, was running a subnormal temperature, and was very weak. He had given a history of gonorrhœa, but denied the knowledge of luetic infection.

There was no means in either case of proving or disproving the reaction given by the blood.

Discussion:

Dr. Park: I asked Dr. MacNeal, who has charge of the Wassermanns in the Research Laboratory, about this question and he said that he did not think that we had diagnosed any case of cancer as syphilis.

Dr. Norris: About two or three weeks ago Dr. Longcope in a meeting at the Academy of Medicine said that at the Presbyterian Hospital 25 to 30 per cent. of the patients gave positive Wassermanns. At Bellevue we found that 40 per cent. of the patients admitted to the wards of Dr. Biggs gave positive Wassermanns. This gives a percentage of patients with positive Wassermanns of between 25 to 40 per cent. of cases admitted without any relation to the clinical diagnosis. The inference is that among this number there must be some cases of cancer.

INFLUENCE OF PROTEIN CONTENT ON ABSORPTION OF ANTIBODIES IN SUBCUTANEOUS INJECTIONS

WM. H. PARK, L. W. FAMULENER AND E. J. BANZHAF

Diphtheria and tetanus antitoxins are supplied in this country most commonly in the form of the refined, concentrated or globulin preparation. These antitoxic preparations contain that fraction of the serum protein designated pseudoglobulin. In the actively immunized horse the antitoxins are most intimately associated with the pseudoglobulin, and, by the process of "salting out," may be separated with it from the other proteins, such as albumin, euglobin, etc. By the most approved methods, a concentrated preparation of diphtheria antitoxin, for instance, may be prepared containing 2,000–3,000 units per cubic centimeter with a protein (pseudoglobulin) concentration of 18–20 per cent. starting with an original serum containing 400–600 units and a protein content of 8–9 per cent. This means of concentration not only greatly reduces the volume as compared with the original antitoxic serum, but at the same time apparently removes certain deleterious substances which are present in the whole serum. Clinical experience has shown that serum reactions are less frequent in patients receiving the refined antitoxin than in those who have had the whole serum product.

In the successful treatment of diphtheria for example, it is a well recognized fact that the specific antitoxin should reach the general circulation in a high concentration as quickly as possible after the onset of the disease. The injection of the antitoxin directly into the blood stream naturally meets such requirements, but owing to the technical difficulties encountered this method is not commonly employed by the practitioner. Instead, the subcutaneous injection is the means of administration in general use. In connection with the subcutaneous method of administration, the question has risen, "Does the highly concentrated antitoxic preparation with its greatly increased protein content prove equally efficacious when compared with a larger volume of serum of lower protein concentration, but containing an equal number of units?" The rate and degree of absorption of the antitoxin from the subcutaneous tissues may be greatly hindered by the high concentration of colloid (protein). This question becomes of special interest in connection with certain commercial preparations of concentrated antitoxin containing a very high percentage of protein.

A series of comparative experiments were undertaken by us in order to test the question of absorption of antitoxin in different protein concentrations. The first experiments were performed upon goats. Nine animals, which were divided into groups of three animals each, of approximately equal weight, were selected for the tests. Each animal received 10,000 units of diphtheria antitoxin by subcutaneous injection in the lower abdominal region. The antitoxin was incorporated with different amounts of protein (pseudoglobulin), and sufficient NaCl was added to make isotonic (0.8 per cent.); together these substances in each combination will be expressed in percentage of total solids. Different volumes of the mixtures also were used in the injections. Two groups of three animals each were done in duplicate; one group averaged slightly heavier in weight than the other. In each group, one animal received a volume of 2.7 c.cm. containing 30 per cent. solids; a second animal, 10 c.cm. volume, 30 per cent. solids, while the third received 10 c.cm. volume with only 7.5 per cent. total solids. As mentioned above each mixture contained 10,000 units of diphtheria antitoxin, and in the cases just cited were injected in one place only. The injections into the third group of three animals were varied from the preceding in that a 30 c.cm. volume containing 30 per cent. solids was substituted in the series for the 2.7 c.cm. injection, while the other injections were quite the same. But instead of injecting in one place, the dose was equally distributed in three places in each animal. Blood samples taken from each animal at intervals after the injection had the serum removed by clotting out, and the antitoxic value of each was determined for the following periods: 18, 36, 48, 72 and 96 hours. As a basis for comparison the antitoxic value in units per c.cm. was computed relative to an animal weighing 50 lbs. and the results tabulated as shown in Table I.

TABLE I

Goats (Subcutaneous Injection)

	10,000 Units Diphtheria Antitoxin.								
	Solids, 30 per cent. (Protein, 29.2 per cent. + NaCl, 0.8 per cent.).						Solids, 7.5 per cent. (Proteins, 6.7 per cent. + NaCl 0.8 per cent.).		
Number	1 H.	2 H.	3 H.	4 H.	5 H.	6 H.	1 L.	2 L.	3 L.
Weight	68 lb.	60.5lb.	57 lb.	47.5lb.	54lb.	46.5lb.	71.5lb.	50lb.	48lb.
Volume injected	30 c.c.	10c.c.	10c.c.	10c.c.	2.7c.c.	2.7c.c.	10c.c.	10c.c.	10c.c.
Places injected	3	3	1	1	1	1	3	1	1
After injection	units	units	units	units	units	units	units	units	units
18 hrs.	7.82	7.86	6.8	5.7	8.37	5.58	8.22	8.75	7.68
36 hrs.	10.2	9.07	8.83	7.6	8.64	6.97	10.72	9.0	8.4
48 hrs.	10.2	8.47	9.12	7.6	8.91	7.16	10.01	8.5	7.92
72 hrs.	8.84	8.47	9.12	7.6	8.64	6.51	9.65	8.0	7.2
96 hrs.	6.8	6.05	7.41	5.7	7.56	6.51	7.86	6.0	7.2

The antitoxic unit strength was first estimated between points of 0.25 units difference. In correcting the figures to a uniform goat weight (50 lbs.) the figures show apparently more accurate estimation.

TABLE II

Men (Subcutaneous Injections)

Injected in One Place	10,000 Units Diphtheria Antitoxin			
	Solids, 18 Per Cent. (Protein, 17.2 Per Cent. + NaCl, 0.8 Per Cent.)		Solids, 9 Per Cent. (Protein, 8.2 Per Cent. + NaCl, 0.8 Per Cent.	
Subject	R——.	S——.	J——.	F——.
Weight	180 lbs.	260 lbs.	125 lbs.	138 lbs.
Volume injected	4.5 c.c.	4.5 c.c.	9 c.c.	9 c.c.
After injection.				
3 hrs.	0.45 units	0.52 − units	0.12 + units	0.27 + units
6 "	0.72 "	0.91 "	0.25 "	0.55 "
9 "	1.17 "	1.04 "	0.31 "	0.82 "
24 "	2.70 "	3.38 "	0.93 "	1.86 "
48 "	3.15 "	4.03 "	2.0 "	2.76 "
96 "	3.15 "	4.29 "	2.0 + "	2.76 − "

The unit value of each serum sample in this table has been computed to a standard weight of 100 lbs. for purpose of direct comparison.

Owing to individual irregularities in absorption, the average values were taken for all the animals which received antitoxin with low solid content, and for those which received antitoxin with high solids, then comparisons were made between the two series. It was found that the degree of absorption was greater for the low solid content mixtures throughout, especially the first period (18 hrs.) in which it was approximately 15 per cent. higher; this difference gradually diminished so that at the 36th hr. period, it was less than 5.5 per cent.; at 48 hrs. 2.7 per cent.; at 72 hrs. 1.3 per cent. but at 96 hrs. 5 per cent.

Shortly after our work was first started, a report of an investigation on the same subject by Walbum appeared from the Danish Government Serum Institute. He drew the conclusion that a high protein content retarded the absorption of antibodies, and considered the use of concentrated antitoxin objectionable from this standpoint. He bases his conclusions upon a series of experiments, carried out upon rabbits with *B. Coli agglutinins,* derived from both goats and rabbits. After completing the series of experiments upon the goats, we decided to do similar experiments upon rabbits using a specific agglutinin, thus taking a different type of antibody and another species for test animal, then compare our results with those of Walbum. A specific agglutinin for *B. typhosus,* derived from an immunized horse, was fractionated and the pseudoglobulin separated with a portion of the agglutinin; this after dialysis was further concentrated in a desiccator over H_2SO_4 at 36°–37° C. The concentrated product contained 20 per cent. solids, including 0.8 per cent. NaCl, and had double the agglutinating value of the original agglutinating serum. The original agglutinating serum contained 9.88 per cent. total solids. Three groups of three animals each were given subcutaneous injections in one place (over the abdomen) as follows: (1) 16 c.cm. original agglutinating serum (low solids); (2) 8 c.cm. volume, conc. agglutinin preparation (high solids); (3) 16 c.cm. volume, composed of 8 c.cm. conc. agglutinin preparation + 8 c.cm. normal pseudoglobulin containing the same percentage of total solids. Bleedings were made at intervals after injection, and the usual quantitative tests for agglutinins were made upon the clotted out serum. In those animals which received the concentrated preparation, a local reaction occurred at the point of injection followed by infiltration, and, in several animals, a necrosis of the surrounding tissues. As is evident, this reaction greatly disturbed the normal absorptive function. However, it may be of interest to note that when the results of the tests were averaged for each of the three groups and curves plotted, they roughly paralleled each other; each group simply showing different degrees of absorption. The first group of animals which received the original agglutinating serum gave only a slight local reaction and showed the highest degree of absorption of the specific agglutinin. The second group which received 8 c.cm. of the conc. preparation showed less absorption throughout, while the third group with the 16 c.cm. volume high solids, was still lower. These results would indicate that an infiltration about the injected area must also be an important factor in retarding the absorption of antibodies.

A third series of tests were carried out upon normal men. Two men received subcutaneous injections of 4.5 c.cm. diphtheria antitoxin containing 10,000 units and 18 per cent. of total solids. Two men were given the same dose of antitoxin (10,000 units) to which an equal volume of physiological salt solution had been added, thus doubling the volume (9 c.cm.) and reducing its percentage of total solids one half. The dose was injected subcutaneously in one place. Bleedings were taken at definite intervals after the injection. The antitoxin content was determined for each blood sample, then the unit value per cubic centimeter was computed to a standard weight (100 lbs.) for comparative purposes. These results are shown in Table II. In these experiments it was found that the degree of absorption was greater in the men who received the concentrated preparation. These results do not accord with those derived from the animal experiments. Considerable variation of weight existed between the different men, the extremes were 125 lbs. and 260 lbs. The weight factor or other individual characteristics possibly influenced the above result; the larger men received the concentrated preparation. Similar experiments are being carried out at the present time upon men with a view of gaining more information upon the weight factor, etc.

The results obtained from our experiments upon animals (goats) and upon men, with diphtheria antitoxin administered with high and low protein concentration, would indicate that absorption from the subcutaneous tissues is variable. The absorption is not greatly influenced by the amount of protein present unless other factors such as inflammatory changes enter; the individual factor apparently plays an important rôle in the result. We must conclude that, from the standpoint of rate and degree of absorption, the refined concentrated antitoxin preparation is practically equal to the whole serum product while in several other respects, it is superior.

NOTE.—Since the above was written more data have been acquired upon the question of antitoxin absorption in man. Two of the subjects, one large and one small, were selected from the group and used in the series shown in Table II. About nine weeks after their first injection each one received a second subcutaneous injection (in one place) of 10,000 units of diphtheria antitoxin from the same lot as used in the first series of tests. But, in this case the smaller man instead of the larger received the 4.5 c.c. volume of 18 per cent. totals solids preparation while the larger man received 9.0 c.c. of the 9 per cent. totals solids preparation. Blood samples were taken from each and the antitoxic content of the serum was determined. Upon comparison, it was found that the absorption of antitoxin in each case corresponded very closely to the results shown in the first test. Therefore, it would appear that the question of absorption in man is independent of the percentage of protein present (within above limits), but is an individual characteristic depending upon the subject receiving the injection.

Discussion:

DR. WADSWORTH: I should like to ask Dr. Famulener if his experiments would show any difference in the length of time that the antitoxin was found in the blood after the injection, and whether the antitoxin persisted longer in one instance than in the other?

DR. PARK: There are a few interesting points which I should like to touch upon. When Walbum reported his work from Madsen's Laboratory, which apparently proved that refined and concentrated antitoxin was much less absorbed than the untreated serum I carefully studied the report to weigh the proof. First, it came out clearly that Walbum had selected his rabbits so as to bring them together in such a way as to get a good contrast. When one changed the rabbits around one obtained a different impression. It is true, however, that the rabbits receiving concentrated serum had the least amount of agglutinin in the blood. He used goat serum coli agglutinin in a rabbit and from that drew the very important and practical conclusion that concentrated horse serum antitoxin should not be used, which is quite an assumption.

Before we used the concentrated antitoxin, we tested it on human beings. We found that absorption took place at least about as readily as with that which had not been concentrated. The charts on the wall show the absorption curve in a number of cases injected subcutaneously, the time for complete absorption being three to four days.

I should like to emphasize the point that in refining antitoxin the elimination of albumins and globulins allows the same protein content in the blood with double the concentration of the units of antitoxin.

Dr. Famulener: I should like to say in reply to Dr. Wadsworth's question that we had not followed out in the normal men the persistence of antitoxin in the blood after injection any further than 96 hours. For instance, the men at 96 hours after injection were running about the level with the 48 hour test so that an estimate of the antitoxin content of the blood was highest about the end of the third day and then slowly declined.

Dr. Park: We found that when there was a local inflammatory reaction the serum was held back and became absorbed later. Toward the later days the antitoxin content of the blood in the cases of slow absorption fell less rapidly than in those in which there was rapid absorption, so that the original difference between the two sets of cases was lessened.

INFLUENCE OF TEMPERATURE AND FLUID MEDIUM ON THE DURATION OF LIFE OF TISSUES REMOVED FROM THE ANIMAL BODY

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A year ago I reported to the Society the results of studies on the effect of cold upon animal tissues, in which the method of cultivating tissues in vitro was used for testing the survival of cells after exposure. It was shown that chick tissues live for several days at temperatures just above the freezing point of the tissue, and that they survive lower temperatures associated with freezing for from a few minutes to several hours, the time depending upon the depth of the temperature. It was concluded, in brief, that the effect of temperatures below the freezing point of the tissue (about —7° C.) is dependent on two factors, depth of temperature and duration of exposure.

Recently, we have been studying more especially the duration of life of tissues kept in several kinds of fluid medium at various temperatures between the freezing point and body temperature, with the object of determining the optimum conditions for preserving tissues outside the body in a state of so-called latent life.

In regard to the influence of fluid medium, the experiments have shown that there is no appreciable difference in the length of life of cells preserved in normal salt solution, Ringer's solution, blood serum and blood plasma,—all isotonic media. In these studies the tissues were kept at 0° and + 6°.

It occurred to us that, although it was found that the kind of medium was a matter of indifference, the quantity of fluid in proportion to tissue might be of importance; a large quantity allowing a dilution of the waste products of cell metabolism. Small fragments of tissue were therefore placed in dishes containing 50–100 c.cm. of Ringer's solution; entire embryos were put into other dishes with just enough Ringer's solution to cover the surface of the tissue. Pieces of tissue taken from both dishes for cultivation in blood plasma at incubator temperature showed that there was practically no difference in the duration of life under the two conditions; indeed, if any difference was observed it was in favor of the tissue preserved with the smaller quantity of fluid, instead of the larger, as our working hypothesis might have led us to expect.

The effect of temperature on the duration of life of tissues in vitro was found to be very definite. The tissues were preserved in small pieces in hanging drops of blood plasma at — 6°, 0°, + 6°, + 15°, + 22°. After varying periods up to eighteen days the pieces were transferred to fresh drops of blood plasma and incubated at body temperature. The following table shows how long the tissues survived at the different temperatures.

Temp.	Duration of life.
— 6° C.	5–7 days.
0°	7–10 days.
+ 6°	11–18 days.
+ 15°	9–12 days.
+ 22°	8–10 days.

It is seen then that at + 6° the tissues survive longest, much longer than at — 6°, a temperature just above the freezing point of the tissue. Remembering that tissues when frozen survive for only a short time, a few hours at most, a curve plotted to show the effect of temperature on the survival of chick tissues would show first a sharp rise, then a gradual incline, then a slow decline. A second rise may be added if we extend the curve to include temperatures around that of the body (30°–40°) at which cells live in a state of active life and growth.

The fact that tissues of the chick and rat embryo may be better preserved at a temperature of + 6° than at lower temperatures is of biological interest, but may be also of practical importance in that it has an application in the preservation of tissues for surgical transplantation.

Discussion:

DR. EWING: In view of the fact that Ehrlich has shown that mouse carcinoma cells are still inoculable after an exposure to extremely low temperatures it seems to me that inoculation into animals affords a better test of the survival of cells than does the method of tissue cultivation in vitro.

DR. PARK: A rather interesting observation on these lines with vaccine virus is that it remains active longer when kept at freezing point and below than at temperatures above freezing. It has kept viable for more than one year when kept at a temperature about — 15° C.

DR. WADSWORTH: The comparatively short viability Dr. Lambert found between 20 and 22° C. is of special interest. A similarly brief viability may be seen among the bacteria. When the bacterial cell is exposed to temperatures just below the thermal limits of growth it is destroyed more rapidly than it is at lower temperatures. For example, the pneumococcus in culture is short-lived and undergoes early autolysis and this is especially marked just above and below its thermal limits of growth. Apparently, when cell growth ceases the cellular enzymes continue their destructive and autolytic activities. At lower temperatures these enzymes are inhibited.

DR. LAMBERT: In reply to Dr. Ewing's remark, I will say that in these studies tumor tissue was not used. However, in other studies in which rat and mouse tumor tissues were used, and in which both animal inoculations and tissue cultures were employed as tests of viability, it has been found that the results with the two tests correspond rather closely. I might say, further, that it is very doubtful if the striking resistance to cold observed by Ehrlich in a single mouse carcinoma will be noted in every transplantable mouse and rat tumor.

PANCREATIC FERMENTS IN INFANTS IN ACUTE INTESTINAL INDIGESTION

ALFRED F. HESS, M.D.

In a former communication which considered the pancreatic secretions in chronic malnutrition in infants it was found that the various ferments of the pancreas are normally secreted even in advanced instances of marasmus or atrophy. In the present study of an acute disease, acute intestinal indigestion, or alimentary intoxication, which was carried out also by the direct method, by the use of the duodenal catheter, the lipase was found deficient, although the two other pancreatic ferments were present in considerable amount. The deficiency of lipase seemed to be to some degree characteristic of this disturbance; it is not a general characteristic of all febrile conditions, and was not met with in pneumonia or empyema. It is possible that the lack of lipolytic activity in this disease should be correlated with the clinical manifestation of fat intolerance, and the metabolic studies showing a deficient absorption of fat.

MIXED TUMOR OF THE UTERUS

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Tumors of the mixed type occurring in the uterus have been reported by a number of observers. Our best treatise on the subject is that of Wilms who in 1903 in his monograph on mixed tumors reviewed the literature of the subject and collected a series of cases dating back to 1867. These had been reported under various names mostly as sarcomata, some as malignant polypi, but all showed more than one type of cells.

The case of Wilms was a tumor of the cervix of polypoid form. Microscopical examination showed areas resembling round-celled sarcoma, areas of smooth muscle showing many giant cells, and areas of hyaline cartilage. Since this case was reported several other such uterine tumors have been recorded, among these, the cases of Penkert, Gebhard, Henziker and Herb. In structure these vary considerably and may show smooth or striated muscle, cartilage, small cysts, sarcomatous tissue, and tissue resembling carcinoma. The body of the uterus may be the site of the tumor. Such is the location of one reported in 1910 by Isabella Herb who was able to find eight other such cases. She notes that they all occurred in married women who had born children and were past the menopause. Those occurring in the cervix are found earlier in life; one, in a child of four years, was reported by Smith in 1883.

As regards malignancy these uterine tumors resemble mixed tumors of the testicle, kidney and vagina in their tendency to recur and to form metastases through the blood stream.

For permission to present this specimen I am indebted to Dr. William H. Bishop. The patient was a married woman of 66 years. There was no history pointing to malignant disease. Clinical diagnosis before operation was uterine fibroid. Panhysterectomy was performed and on account of adhesions 16 inches of the descending colon had to be resected. A Murphy button was used for the anastomosis and six weeks later had to be removed by operation. The wound broke down and death from exhaustion occurred at the end of ten weeks. Autopsy could not be obtained.

The uterus is globular, measures 11 cm. in length and 9 x 6.5 cm. at fundus. Surface smooth except at fundus, where there is a small protrusion of soft friable tissue. Outline of cervix lost and in region of external os there is a dark red protrusion which resembles a blood clot.

On section there is a solid tumor mass of globular form surrounded by a capsule formed by the uterine muscularis. This is thinned out to .5 cm. or less and at fundus has been penetrated by the tumor. The growth is soft in consistency and shows necrotic and hemorrhagic areas. No uterine canal can be made out. The gross appearance is very similar to the illustration of Penkert's mixed tumor but there was not the same polypoid appearance in his case. The growth appears intimately connected with the muscularis throughout.

The Fallopian tube is enlarged, surface rough and on section cavity shows dark red tissue. Ovary, slightly enlarged.

Microscopically, the growth consists, (1) of areas of large spindle cells with abundant chromatin in the nuclei with scattered larger oval or round cells or giant cells—the general picture of an actively growing spindle-celled sarcoma. Many of the cells show mitosis;

(2) Of large irregular alveoli lined by cuboidal epithelium and surrounded by connective tissue stroma or sarcomatous tissue;

(3) Of masses or nests of round or oval cells with the appearance of a carcinomatous growth;

(4) Of scattered islands of hyaline cartilage.

Sections from the region of the cervix show much necrotic tissue and hemorrhagic areas. Careful search fails to show any striated muscle fibres although many of the reported cases contain much of this tissue. Henziker calls his tumor a rhabdomyoma and cites six of this type.

Cross section of the tube shows blood clot and an area of tumor cells which suggest a carcinoma metastasis. There are also islands of hyaline cartilage.

The ovary is invaded by new growth which has the type of an adeno-carcinoma.

The gross and microscopical characteristics of the tumor seem to place it among the mixed celled tumors of the body of the uterus.

THE ARTIFICIAL PRODUCTION OF STRUCTURAL ARRESTS AND RACIAL DEGENERATION

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Various chemical and physical means are often experimentally employed to modify development. In fact the development of an animal embryo in almost any unusual or abnormal environment causes the embryo to respond by deviating from the normal path of development to a degree depending upon the extent of the environmental modification. The same method of treatment does not cause every embryo to respond in a like manner, so that when a number of developing eggs of a given species are subjected to certain chemical stimuli numerous types of defects result. It has been possible, however, to regulate the treatment in certain cases so as to induce a rather definite or consistent response on the part of the embryo.

The developing eggs of two species of fish when subjected to the actions of various magnesium salts, alcohols, ether, etc., have been found to respond by giving rise to large numbers of cyclopean monsters. This response is oftentimes most consistent, so that sixty in one hundred individuals will be of the cyclopean type. Other defects of the eyes and central nervous system are also caused to occur in these solutions. All of the eye conditions may be interpreted as arising through developmental arrests. The abnormal environment tends to lower the developmental vigor of the embryo and this lack of vigor is particularly shown in the condition of the eyes, since the budding off and outpushing of the optic vesicles are apparently the most extensive growth phenomena taking place in the early embryo.

The embryos treated with alcohols, etc., often had abnormal brains and in addition to cyclopia, which was the most frequent eye defect, many were completely eyeless, while others had one eye of the pair fairly well developed while the opposite eye was small and defective or at times entirely absent. Such conditions were termed monophthalmica asymmetrica.

The eggs of the common fowl may be treated with the same substances as those above mentioned. The developing embryos respond in a somewhat similar manner, though the response is not so consistent as in the fish. When hens' eggs are placed in an atmosphere saturated with the fumes of alcohol, within a short time, from one to three hours, enough fumes penetrate the shell and act on the early embryo to cause it to develop abnormally. Many of the affected chicks showed eye conditions similar to those produced in the fish. Cyclopia was not uncommon, and the monophthalmic asymmetrical monsters were often produced.

The cause of these defects in the chick is thought to be the same as in the fish. The fumes acted to weaken or injure the embryo so that it no longer possessed sufficient developmental energy to give rise to normal fully formed optic vesicles. In some cases the arrest prevented the lateral migration of the vesicles so that they arose in a ventro-median position giving rise to cyclopean monsters. Other individuals were able to separate the eye anlagen, yet one of the anlagen failed to differentiate normally and so formed an imperfect eye or was entirely suppressed, thus no eye or a defective eye occurred on one side. Still other specimens were completely unable to differentiate or develop the eye parts and gave rise to eyeless chicks.

It is thus seen that in fishes and birds the experimenter is able to regulate the type of abnormality to some extent at least. The eggs of these animals develop externally; that is, outside of the maternal body. Such eggs may be directly treated with various substances. The eggs of higher mammals develop within the mother's body and the proposition of treating them with chemical or physical stimuli is thus complicated. The question arises whether the effect is due to the action of the substance used upon the embryo directly or whether the effect is secondary and due to the changed condition induced upon the mother by the treatment.

It is found that volatile substances such as alcohol and ether may be employed with the least complication in their manner of action. When alcohol is given by inhalation to guinea pigs it is readily taken into the animals' blood and produces a state of intoxication. Guinea pigs given alcohol in this way six times per week have maintained their bodily vigor, fattened and lived in an apparently healthy state for as long as two and one half years.

Other investigators have found that alcohol given to pregnant guinea pigs passes directly into the blood and tissues of the fœtus. It has also been shown that alcohol has an affinity for the reproductive glands and may be demonstrated in the seminal fluid of the male shortly after being administered. Thus the spermatozoa may practically be bathed in a solution of alcohol, and the early embryo of a mammal may be subjected to the action of alcohol almost as directly as the embryo of the fish or hen considered above.

The guinea pigs were first tested by normal matings and found to produce vigorous offspring. Matings of the alcoholized guinea pigs were then made in various combinations. Alcoholic males were paired with normal females. This is the paternal test and is the only true test of the action of the substance on the germ cells. In such matings the egg is from a normal female and develops in a normal body, hence any defect in the offspring is probably due to the action of the alcohol on the spermatozoon.

Treated females were paired with normal males, the maternal test; in this mating there are two chances of affecting the offspring. The alcohol may have acted on the unfertilized ovum so as to modify its nature and cause it to develop into an abnormal individual or secondly the ovum may be normal but its development within a mother with alcoholized blood may result in a defective guinea pig. In any case we would be unable to differentiate between these two causes, yet the modified condition is no doubt often due to both. The mammalian mother has two chances to injure an offspring, either by producing a defective egg, or by supplying an unfavorable or diseased environment in which the embryo must develop.

The final combination is the mating of two alcoholic individuals; this, of course, offers the greatest chance for defective offspring.

Effects of Alcohol on Offspring of Treated Animals

Condition of the Animals	Number of Matings	No Result or Early Abortion	Still-Born Litters	Number of Still-Born Young	Living Litters	Young Dying Soon After Birth	Surviving Young
Alcoholic ♂ × Norm. ♀	53	22	7	13	24	20	31
Norm. ♂ × Alcoholic ♀	14	2	3	9	9	9	10
Alcoholic ♂ × Alcoholic ♀	27	14	3	6	10	5	9
SUMMARY.	94	38	13	28	43	34	50
Control Norm. ♂ × Norm. ♀ ..	33	0	1	4	32	4	56
Results of Mating Second Generation— Untreated							
2d Gen. × Normal.	2	0	0	0	2	0	3
2d Gen. × Alcoholic	2	0	2[1]	5[2]	0	0	0
2d Gen. × 2d Gen.	13	4	0	0	9	4[3]	10

[1] 2n ♀ × a ♂; 2n ♂ × a ♀.

[2] 1 Det.

The accompanying table shows the results obtained in 94 matings of the treated animals. The upper line shows the effect of mating alcoholic males with normal females. In 53 such matings 22 gave early abortions or no results, while control matings invariably gave offspring. Seven stillborn litters occurred among the 31 productive matings. Twenty-four living litters were born, consisting of 51 individuals. Twenty of these young animals died within a few days showing various nervous conditions and usually dying in convulsions.

The second line gives similar results when normal males are mated with alcoholic females. The injurious effects shown by this combination are only slightly more marked than in the above case, where the male alone was treated.

The next line indicates the result of the combination between alcoholic males and females. At this stage of the experiment the results shown here are little if any worse than those in the above cases.

A summary of the outcome of the matings of alcoholic individuals may be expressed as follows: out of a total of 94 matings 38, or more than 40 per cent., were negative or resulted in early abortions, the mother often eating the embryos, so that they could not be definitely recorded. Thirty-three control matings never failed to give results. Of the 56 alcoholic matings which gave results 13, or 23 per cent., were stillborn litters while 43 were living litters which consisted of 84 individuals, of these 40 per cent. or 34 individuals died within a few days.

The 33 control matings gave rise to only one stillborn litter, in 32 living litters of 60 individuals only 4 young, or 6 per cent., died. The results of these matings stand in striking contrast to those of the treated animals.

The lower part of the table gives the data obtained from the few combinations which have been made up to the present time with the offspring from the treated animals. These we may term the second generation. The animals themselves have not been treated although they were derived from treated parents.

In 2 cases second generation animals were mated with normal individuals and the results were perfect—two living litters were born consisting of three young. If one may speak from so few cases it would seem as though the normal mates had counteracted any weakness that may have been present in the other animals.

When two second generation guinea pigs were mated with two alcoholic animals the results were almost disastrous. Two stillborn litters were produced, consisting of five fœtuses, one of which was deformed. It must be recalled that in the above section of the table the 27 matings made between animals both of which were alcoholic gave in all only 3 stillborn litters.

Thirteen matings of second generation males and females gave negative results in 4 cases, or about 30 per cent., nine living litters were born consisting of 14 individuals, four died within a few days after birth, one of which was deformed, and ten survived. It will be noticed that two deformed young have occurred in these 17 second generation matings while in the offspring resulting from the 127 matings above no deformity was observed.

One of these deformed specimens was entirely eyeless. This is of interest in connection with the experiments on the fish and bird, described above. It would seem as if this alcohol treatment had produced a weakened race. The embryos developing in such a mother have insufficient developmental vigor to form a perfect nervous system and in the one case the eye anlagen were completely unable to arise or differentiate so that an eyeless individual resulted. The optic nerves and tracts are, of course, absent. This eyeless guinea pig had an alcoholic grandfather, its maternal ancestry consisted entirely of untreated animals.

Random Group of Matings.

2n Nov. 4—100♀ × 92♂ = 0.
2n Nov. 4—101♀ × 99♂ = 0.
2n Nov. 15— 91♀ × 93♂ = Feb. 18—Two apparently normal offspring.
2n Nov. 15— 98♀ × 95♂ = 0.
2n Nov. 16— 76♀ × 77♂ = Jan. 28—Three offspring died few days, one deform.
2n♂ Nov. 16— 88♀ × 78♂ = Feb. 20—One normal, long gestation.
2n♂ Nov. 16— 87♀ × 75♂ = Jan. 27—Two small offspring.
♂ Nov. 16— 66♀ × 5♂ = 0.
♂ Nov. 16— 19♀ × 6♂ = Jan. 30—Three, two died at birth, one weakling.
♂ Nov. 16— 34♀ × 43♂ = Feb. 4—Two apparently normal.
♂ Nov. 16— 49♀ × 45♂ = Jan. 31—Three apparently normal.
C Nov. 16— 33♀ × 46♂ = Jan. 30—Two large offspring.
C Nov. 23— 50♀ × 69♂ = Feb. 5—Two large offspring.
C Nov. 23— 15♀ × 70♂ = Feb. 4—Two large offspring.
C Nov. 23— 54♀ × 72♂ = Feb. 3—Two large offspring.
C Nov. 23— 56♀ × 80♂ = Feb. 3—Three large offspring.
C Nov. 23— 52♀ × 81♂ = Feb. 12—Two large offspring.
C Nov. 23— 53♀ × 48♂ = Feb. 3—One very large offspring.

The next table shows a random group of eighteen matings and gives an idea of the individual responses obtained during the experiments which have now been conducted for two and a half years. These represent the last November matings. Four of the 18 gave negative results, one of these was a double alcoholic mating, at this stage of the experiment this mating generally fails to give offspring. The other failures were from the second generation matings. These failures are not due to the fact that the animals are inbred or that they do not continue to breed in the laboratory. Some of the control matings are from second and third generations kept under identical conditions.

It is hoped that these experiments may be further regulated so as to give more definite responses of the nature obtained with the lower forms, fish and birds. At the present they show that such treatments tend to lower developmental vigor and give rise to weak and more or less degenerate types of offspring.

These experiments are the first to show in a definite manner that the mammalian offspring may be injured or modified in its development by treating the male parent so as to affect his germ cells.

For a more detailed account of most of these experiments the reader is referred to *Jour. of Exper. Zool.*, 1906, III, 99; same, 1907, IV, 165; same, 1909, VI, 285; *Arch. f. Entwicklungsmech.*, 1907, XXIII, 249; same, 1912, XXXV, 569; *Am. Jour. Obst.*, 1909, LIX, No. 4; *Am. Jour. Anat.*, 1910, X, 369; *Arch. of Internal Medicine*, 1912, X, 369.

PATHOLOGICAL FINDINGS IN AN EPIDEMIC OF RAT TYPHOID

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Interest in epidemics amongst rats and mice dates back to the publications of Loeffler in 1890. Loeffler isolated from an epidemic in laboratory mice a Gram-negative, motile bacillus of the colon-typhoid group, to which he gave the name *B. typhi murium*. He used with great success cultures of this organism to combat a plague of field mice which were ravaging the fields of Thessaly, causing great destruction of the crops. Since this first successful experience, and especially since the growth of our knowledge concerning the role of the house rat in the transmission of plague, many organisms of this colon-typhoid group have been isolated, either from spontaneous epidemics of rats or mice, or from other sources, and applied on an extensive scale to the destruction of rats. The more familiar of these so-called "Rattenschädlinge" are the Danyscz Bacillus, the Issatschenko Bacillus used in Russia, the Liverpool virus, the Ratin virus from Copenhagen; a number of other strains supplied commercially or prepared by various hygienic institutes have been studied.

The interest in the study of these organisms has largely centered upon their practical use in the extermination of rats and mice. Many careful bacteriological studies have shown the identity of some, if not all, of these strains, with the Gärtner bacillus, and the possibility that their indiscriminate use might give rise to epidemics among human beings has in several instances been established.

In contrast to the abundant literature on the bacteriological and hygienic aspects of the disease is the scarcity of reports bearing upon the pathological findings. Indeed, a detailed study of the microscopic lesions has, so far as I am aware, not been made. I have been informed that Dr. Mallory several years ago was impressed by the resemblance of the lesions to those of human typhoid, and that one of his assistants is at present engaged in the study of the disease from the pathological point of view. Since their work has not yet appeared, it seemed of interest to present a brief report of some observations made by Dr. von Wedel and myself during an epidemic which broke out among the white rats in our laboratory. Our studies are not yet completed and will appear in detail elsewhere.

We have no data as to the percentage of mortality. During the winter months the deaths were very numerous, but at present the epidemic seems to have spent itself and of late no rats have become spontaneously affected. The duration of the disease, as it occurred spontaneously, could not be determined but appeared to vary within wide limits. In several litters which had been under observation for some time and which were being weighed daily, the duration of the disease as measured from the initial loss of weight was 3, 4, 7, 10 and 12 days respectively. These data are only approximately accurate, as the animals were killed when apparently moribund. Moreover, in two rats characteristic microscopic lesions were found, although there had been no previous loss of weight.

In animals experimentally infected and allowed to die, the duration of the illness varied with the mode of infection. Emaciation was very marked in most of the rats. There was regularly observed a marked anemia as judged by the ears which became blanched, and the pallor of the eye grounds. Smears from the blood showed great numbers of normoblasts, and marked polychromatophilia and granular degeneration of the red cells. The normal pink color of the eyes was often changed to a brownish or chocolate tinge by which the disease could be easily diagnosticated. This has been shown by Boycott to be due to methhæmoglobinæmia.

A bloody crust about the eyes and nares was always present in the infected rats, especially in the terminal stages. Diarrhea occurred in some but by no means in the majority of the cases. Many of the rats showed no intestinal disturbances and voided normal dry globular scybalæ until their death.

Gross Lesions

The animals were more or less emaciated. The peritoneal cavity was free from exudate, even in animals killed by intraperitoneal inoculations. The liver was large, pale, friable, and sometimes mottled. The necroses, regularly found on microscopical examination, were not visible in the gross, although with a magnifying glass there could sometimes be seen opaque points. The spleen was very greatly enlarged in all cases. Thus in one litter, the spleen of the infected animal measured 36 mm. in length as against 33 mm. and 32 mm. in the healthy controls. In another litter, the infected spleens measured 37 mm., 40 mm., and 39 mm.; that of a healthy animal of the same litter, killed at the same time, 23 mm. The spleens were firm and tense, very dark bluish purple but irregularly mottled on section by hemorrhagic and grayish areas. The intestines were usually collapsed; in those animals which had previously shown diarrhea, the intestinal contents were fluid, pale and occasionally streaked with blood. Marked swelling of the Peyer's plaques, such as has been noted by previous workers in similar epidemics, was rarely seen by us; nor was there a noticeable hyperæmia, either of the intestine or of the peritoneal vessels. In one animal, inoculated subcutaneously, several small ulcers were found in the Peyer's patches. No exudate was ever found in the pleural or pericardial cavities. The lungs, with the exception of occasional small purpuric spots beneath the pleura, showed no gross changes. The heart muscle was sometimes rather pale, and in one spontaneously infected rat, showed macroscopically visible opaque grayish areas in the left ventricle.

Swelling of the cervical and mediastinal lymph-nodes was quite regularly found. No gross changes were noted in the kidneys, adrenals, pancreas, testes or brain.

Microscopic Lesions

The *liver* in both spontaneously and experimentally infected rats was the seat of numerous focal necroses. These were present in all the cases examined, with the exception of three rats which were killed on the day following inoculation. The smallest and earliest lesions (30 hours after infection) appeared as agglomerations of distorted nuclei, lying in a fibrinous meshwork in which are entangled a few red blood cells. In the older lesions, the nuclear constituents gradually disappear; there remains a sharply outlined, usually circular area, almost devoid of cells, staining intensely with eosin, and showing a swollen nodular fibrinous framework. In places the interlacing nodular strands of fibrin appear to fuse into hyaline masses. The fibrinous material stains orange red with Mallory's connective tissue stain, and bluish with Weigert's fibrin stain.

The necrotic areas are at once made evident, in sections stained with Sudan, by the abundant accumulation of fat droplets. These are of largest size and most numerous at the margin of the necroses. The central portion may contain a few finely divided droplets, or may be virtually free from fat. Here and there are small isolated groups of liver cells laden with fat. These are probably at the edge of necrotic areas which did not appear in the section. Fine droplets are also found free in the capillaries and within the Kuppfer cells.

The liver cells apart from the necrotic areas show no degenerative changes. The cytoplasmic granulæ are distinct. In some of the rats, notably in one killed 24 hours after subcutaneous inoculation, mitoses are very numerous, but not especially so about the necroses.

Within the capillaries are found in great numbers abnormal cellular elements and occasional fibrin thrombi. The cellular structures are for the most part large mononuclear cells in various stages of disintegration; they usually have large irregular lobate nuclei and enclose in their cytoplasm nuclear fragments; sometimes the fairly preserved nucleus of a small lymphocyte, and sometimes a red blood corpuscle. One finds also unphagocyted chromatin fragments, and small cells with ring or signet formed nuclei,—obviously lymphocytes in various states of pycnosis. In some of the sections cellular elements and detritus of this sort are so abundant as virtually to block the capillaries. They are found in large masses in the portal veins.

The Kuppfer cells themselves, though occasionally swollen and sometimes exfoliated, are apparently not the chief source of these foreign cellular elements in the liver capillaries. This could be shown fairly definitely by staining an infected animal with Trypanblau. Very few of the cells lying free in the capillary lumen contain blue granulæ. There is no especial accumulation of the stained cells about the necroses, or within them. Those endothelial cells which lie inside of the necrotic areas are obviously degenerated; the blue staining material is in the form of large clumps instead of granulæ of fairly uniform size.

As regards the fibrin thrombi, these are quite numerous in some sections. They are club-shaped on longitudinal section, with one end attached to the capillary wall. Exfoliated endothelial cells and red blood cells lie upon their surface. Sometimes the adjacent liver cells show a change in staining reaction, with vacuolization of the protoplasm and hydropic swelling of the nuclei. There may be extravasation of red cells between the liver cells.

Spleen: In this organ the lesions are localized in the early stages, more diffuse in animals which survive the infection for a longer period. The essential change appears to be an intense destruction of lymphoid cells, both within and without the follicles. Throughout the pulp one finds small mononuclears in all stages of disintegration as evidenced by the abundance of fragmented chromatin. Much of this nuclear detritus is within macrophages. The necrosis of the pulp, which in the earliest stages is focal but later involves almost the entire pulp, is accompanied by a great outpouring of fibrin which forms a nodular network; the swollen filaments appear to use the fibrous reticulum as a support. There is often extensive hemorrhage. No fibrin could be demonstrated within the follicles. The reticular cells of the follicles are swollen, and often phagocytic towards the pycnotic lymphoid cells. Mitoses are frequent in the early stages.

Bacilli are found in small groups or in larger clumps both within and outside of the sinuses.

Kidneys: Hyaline thrombi were frequently found in the glomerular capillaries. This lesion was especially striking in several of the experimental animals, in which every tuft showed a more or less complete blocking. The appearance was especially distinct in sections stained with Mallory's connective tissue stain, the hyaline material taking a reddish orange color in contrast to the yellow of the red blood cells.

Bacterial emboli in the glomerular loops and in the capillaries between the tubules were seen in two rats inoculated intraperitoneally, but were not regularly present.

Myocardium: Lesions in the myocardium were found in both the spontaneously and experimentally infected rats, though not in all cases. The changes were in the nature of an acute interstitial inflammation, and occurred by preference in the superficial strata of fibers, more especially in the left ventricle. Rat *A* (spontaneous illness) showed extremely marked lesions. Near the apex, the muscle fibers are forced apart by cellular exudate and fibrin. Most of the cells are fragmented and distorted beyond recognition, but at the margin of the lesions it can be seen that there is swelling and degeneration of the fixed connective tissue cells, as well as a moderate exudation of lymphoid and polynuclear elements. The muscle fibers show various degrees of degeneration up to complete necrosis. In some areas, the fibers have disappeared completely, leaving only an irregular eosin-staining material in which are found swollen distorted nuclei and chromatin fragments, apparently derived from the breaking up of the leucocytes. Bacilli, occurring singly and in small clumps, are found without difficulty between the muscle fibers.

Lungs: The septa appear relatively stout and more cellular than those of normal lungs. This is due to the stuffing of the capillaries with large mononuclears and cell fragments similar to those found in the liver. Many of the larger pulmonary arteries contain groups or larger masses of these cells. Pneumonic lesions were not found, and the bronchi were free from exudate.

Stomach and Intestines: No microscopic lesions were found in the stomach. The intestinal changes were not striking, and in some of the animals, no definite lesions were found. In others, there was moderate fragmentation of the lymphoid cells, with phagocytosis by the swollen reticular elements. The overlying mucosa was intact. A recently experimentally infected animal, which showed marked swelling of the Peyer's plaques with several definite ulcers, has not yet been examined microscopically.

Serial sections of the neck organs were made from a number of the spontaneously infected rats for another purpose. In all the cases, it was found that the cervical lymph-nodes were greatly enlarged and the seat of extensive necroses similar to those described in the spleen. Although the *thymus* exhibited advanced involutional changes such as are found in all infectious diseases accompanied by wasting, in no case did it contain areas of necrosis, even when all the surrounding lymphoid tissue, including that which abundantly envelops the larger bronchi, was extensively affected.

The *marrow* of the long bones contained more or less extensive areas of necrosis distributed throughout the shaft. Necroses were also found in the bones of the jaw and in the clavicle.

No lesions were found in the *thyroid, parathyroid, pancreas* or *testes*. The *adrenals* showed no loss of chromaffinity and were normal in other respects. The *nervous system* has not yet been studied.

It will be seen from this incomplete report that the lesions bear some resemblance to those of human typhoid, save that the intestinal lymphatic tissue is less conspicuously injured in the rat disease. Of particular interest from the pathologist's point of view is the opportunity which the disease affords for the experimental study of focal necroses. The exact manner in which these are produced in human typhoid fever is still a matter of dispute. One point to be determined is whether the necroses arise from the toxic destruction of liver cells, as a direct or indirect effect of the bacterial poison; or, as Mallory holds, from the embolic occlusion of liver capillaries by macrophages or "endothelial leucocytes" swept out from the spleen and intestine. Although we have not solved the problem definitely, the following observations seem to have a direct bearing upon the question, and with certain reservations, tend to support Mallory's view that occlusion of the capillaries is the primary cause of the necroses. Bacilli are only exceptionally found in the necrotic areas, so that there is little reason to believe that the destruction of the liver cells is brought about by the local action of the organisms. Large cells of various types, often with phagocytic inclusions, are found in great numbers in the liver capillaries and in many places appear to fill up completely the lumen. About these masses of impacted cells there are formed fibrin thrombi. By injecting India ink into the portal vein, it can be clearly shown that even the smallest necroses are impervious to the injection fluid. Furthermore, by comparing smears made from the splenic blood with those made directly from the heart, it can be shown that a large proportion of the degenerating mononuclear macrophages are filtered out by the capillaries of the liver and lung.

We are at present engaged in a further study of this problem and hope to arrive at a final solution. The detailed report of our study with a description of the lesions obtained in other susceptible animals will appear later.

A BACTERIOLOGICAL STUDY OF A RAT EPIDEMIC

H. VON WEBEL, P.D.

(From the Department of Bacteriology of College of Physicians and Surgeons.)

A bacterial investigation was made of three rats dying of spontaneous infection during this epidemic. All the organisms obtained in pure culture were compared morphologically and biologically to determine their identity. These pure cultures were then experimentally inoculated in four rats and fed to two others in order to fulfill Koch's postulates for establishing the etiological relationship of a microorganism to a disease.

The first rat, which we numbered A1, had a profuse diarrhœa and there were crusts of blood around eyes and nose. These symptoms were noticed in many of the rats that had died during the epidemic. At autopsy, from the heart, liver and spleen pure cultures were obtained of a Gram-negative bacillus. This was slender and actively motile, resembling morphologically the typhoid bacillus.

The second rat, A2, was autopsied about six hours after death. From the heart blood, liver and spleen, in all plates, pure cultures were obtained of an organism which compared morphologically and biologically in all its characters with the Gram-negative bacillus found in rat A1. Similarly the third rat, A3, was autopsied and pure cultures of this organism were again obtained from the heart-blood, liver and spleen. Other rats were examined during the epidemic and Gram-negative organisms were found in all, but we did not examine these organisms culturally.

The organism was easily isolated in pure culture from all the rats examined. It is a slender, actively motile, Gram-negative, non-sporebearing bacillus, which stains with the ordinary laboratory dyes. In the tissues of the rat the bacillus appeared much larger than in smears from cultures and also showed bipolar staining. The thermal death of the organism was 57° C. after 10 minutes exposure in broth. On agar plates the organism grew rapidly, forming moist gray-white colonies which were large and round with smooth borders. On agar slants the growth had the same gray-white moist appearance. The water of condensation was cloudy with a heavy sediment for the first few days; after standing for a week this cleared. Gelatine stab cultures developed as a fungiform growth and were not liquefied in 30 days.

Broth was evenly clouded in 24 hours. Afterwards a heavy sediment and a thin pellicle formed. At the end of four days the sediment collected in large flakes at the bottom of the tube.

Nitrates were reduced to nitrites.

In Dunham's peptone broth there was a slight indol production after four to six days growth in nearly all tests.

On potato a scant gray-white growth was obtained that became visible only after three to four days.

Litmus milk at the end of twenty-four hours was acidified, at the end of forty-eight hours neutral, and at the end of three days alkaline. It was not coagulated.

The organisms isolated from the three rats were compared with cultures of a bacillus isolated from the spleens of mice at the Rockefeller Institute, cultures of *Bacillus typhi murium* from the Natural History Museum and from our laboratory collection. The strain from the Natural History Museum was originally isolated by *S. Miggi* from the "Liverpool virus," a commercial rat poison sold here. These comparisons were made on serum water media and in fermentation tubes with tested sugars in sugar-free broth, with the results tabulated on this chart:

Sugar Fermentation Reactions

Strains	Rat No. A1	Rat No. A2	Rat No. A3	Rockefeller Institute Bacillus	N. H. Museum *B. typhi murium*	Laboratory *B. typhi murium*
Dextrose	Acid	±	Acid	++	+	+++
Maltose	+	+	+	++	+	++
Levulose	+	+	+	++	++	++
Lactose	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline
Mannite	+	+	±	+++	+	++
Saccharose	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline
Dextrin	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline
Galactose	+	+	+	++	+	++

Note.—Sign + indicates gas production in fermentation tubes.

According to these morphological and biological characteristics the organisms obtained from each of the three rats correspond accurately with each other and with the strain of the *Bacillus typhi murium* obtained from the Natural History Museum. The other two strains tested corresponded in their cultural characteristics including their fermentations but the gas formation was more marked. Finally we compared the agglutinability of our three strains of organisms with strains of *B. typhosus, B. para typhosus* (Schottmüller), *B. enteritidis* (Gärtner), *B. typhi murium* from our laboratory and from the Natural History Museum and the bacillus from the Rockefeller Institute. The results are tabulated on this chart:

The three strains of our organism isolated from the three rats 1 A, 2 A, 3 A, and the culture of the *Bacillus typhi murium* obtained from the Natural History Museum collection correspond accurately in their agglutination reactions; but the culture of this organism obtained from the laboratory collection failed to agglutinate. The bacillus isolated from mice at the Rockefeller Institute also failed to agglutinate. *B. Enteritidis* agglutinated in dilution of 1–160, and the *B. typhosus* in dilution of 1–1000, whereas the *B. paratyphosus* failed to agglutinate.

In order to establish the etiological relationship of the organism to the disease we inoculated six rats brought into the labora-

Agglutination with Serum of Rabbit Immunized with the Bacillus from Rat 2A

Strain	Rat B, 1A			Rat B, 2A			Rat B, 3A			N. H. M. *B. typhimurium*			R. I. Bacillus			Laboratory *B. typhimurium*			*B. enteritidis* Gärtner			*B. typhosus*			*B. paratyphosus* (Schottmüller)		
Time in hrs.:	½	2	4	½	2	4	½	2	4	½	2	4	½	2	4	½	2	4	½	2	4	½	2	4	½	2	4
1–20	+	++	+++	+	++	+++	+	++	+++	+	++	+++	–	–	–	–	–	–	–	±	+	±	++	+++	–	–	–
1–40	+	++	+++	+	++	+++	+	++	+++	+	++	+++	–	–	–	–	–	–	–	±	±	±	++	+++	–	–	–
1–80	+	++	+++	+	++	+++	+	++	+++	+	++	+++	–	–	–	–	–	–	–	–	±	±	++	++	–	–	–
1–160	+	+	++	+	+	++	+	+	++	+	+	++	–	–	–	–	–	–	–	–	±	±	++	+	–	–	–
1–200	+	+	++	+	+	++	±	±	++	+	+	++	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
1–500	+	+	++	±	+	++	±	±	+	+	+	++	–	–	–	–	–	–	–	–	–	–	±	+	–	–	–
1–1,000	±	±	+	±	±	+	±	±	+	±	±	+	–	–	–	–	–	–	–	–	–	–	–	±	–	–	–
1–2,000	–	±	+	–	±	+	–	±	±	–	±	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1–4,000	–	±	±	–	±	±	–	–	±	–	±	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1–8,000	–	–	±	–	–	±	–	–	–	–	–	±	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Saline control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

tory especially for these tests from a lot that had no record of disease among them. They were kept under observation for two days before they were used.

Two rats were inoculated intraperitoneally with 0.5 c.c. of a saline suspension of the bacilli grown on agar for 24 hours. These rats died in two and three days. They developed no characteristic symptoms.

Two others were inoculated subcutaneously with the same amount of bacterial suspension. They had no diarrhœa but bloody crusts around nose and eyes formed in five days and the animals died in six and seven days.

The last two were fed with bread soaked in a 24-hour broth culture. They developed all the characteristic symptoms and died in nine days.

At autopsy the characteristic lesions were present in all three experimental tests, and the organism was recovered from the heart blood and organs.

These results therefore clearly established the etiological relationship of the organism to the disease.

In order to determine whether or not the bacillus was pathogenic to other animals we inoculated a rabbit, a guinea-pig, and two mice, one intraperitoneally and one subcutaneously with a saline suspension of the bacilli. 1 c.c. of the saline suspension killed the rabbit in three days. 1 c.c. killed the guinea-pig in 18 hours and ⅓ c.c. killed the two mice in 18 hours. In every case the organisms were recovered in pure culture, and in smears from the heart blood of the animals large numbers of bacilli were found showing that the organism had developed giving rise to a true infection. It was difficult to find bacilli in smears of the heart blood of the rats dying of spontaneous infection although they grew in cultures. We also inoculated a rabbit and one mouse with the same amounts of the bacterial suspension, which had been given to the other animals, killed by heat, to determine the toxicity of the material inoculated and as a control for the virulent inoculated material of the cultures. The rabbit and the mouse are still alive. As yet we have not tried to infect other animals by feeding.

The majority of workers, however, have not been able to reproduce the disease in rabbits, cats and dogs by feeding them with the bacillus isolated from epidemics in rats. The work of Mühlens, Dahm, Fürst, Van Ermengen, Schern, Steffenhagen and others suggest that both in their cultural characteristics and their agglutination reactions the organisms they isolated from rat epidemics agreed with the Danysz, Ratin, Issatschenko, and Gärtner bacillus, and also with the Liverpool virus. But it is evident that the bacillus we have isolated from the epidemic differs slightly in the degree of its gas production and markedly in its agglutination reaction from all the strains of this group of organisms with which we have as yet been able to compare it, except in one notable instance, the *Bacillus typhi murium*, which we secured from the Natural History Museum and which was originally isolated from the "Liverpool virus," a commercial rat poison sold in England and in this country.

This Liverpool rat virus has also been investigated by Handson, Williams and Klein. An epidemic broke out in a large business house in London. Twelve persons were taken ill with the disease. Ten days later when they were all convalescent an investigation revealed the fact that although these men all ate at the business house with many other employees, only those who ate in a certain room were taken ill. In this room, upon removing the floor boards, 40 dead rats were found! It was then discovered that a rat poison, the Liverpool virus had been spread on bread and placed around this room so that the rats could get it. Cultures were made from the dead rats, and from the patients, and Klein reports that the bacilli isolated agreed in every respect with each other and with the organism found in the Liverpool virus. Blood sera of the convalescent patients agglutinated the organism of the "Liverpool virus," the organism isolated from the dead rats and the organism isolated from the patients.

The immune sera of our organism fully agglutinated only one organism, namely, the Natural History Museum strain which was obtained from the "Liverpool virus." Making deductions from this it would seem that the organism which we isolated from the rats agreed in all tests with the organism of the "Liverpool virus" and that the use of these commercial rat poisons containing virulent organisms may be a menace to man.

Discussion of papers by Dr. Pappenheimer and Dr. von Wedel:

Dr. Wadsworth: This communication is of particular interest to the bacteriologist in two respects. First, the difficulties experienced in attempting to identify this organism in a fairly well-known group showing that although the organism corresponded in all its cultural characteristics with the other members of the group which were studied the agglutination test revealed marked differences in the reactions of the different strains save in the tests with the one strain of *B. typhi murium* obtained from the Natural History Museum. These results illustrate very clearly the difficulties in the present methods of the identification of species. Then, the relationship of this organism to the commercial rat poison I think is one of considerable importance, although in the London epidemic none of the cases died, they evidently were seriously ill and certainly the sale of a commercial rat poison with living organisms should be considered a menace to man, as Dr. Pappenheimer and Dr. von Wedel have pointed out.

Dr. MacCallum: Since necroses may be produced in the liver and studied in all stages it should be possible with this infection to determine the validity of Dr. Mallory's ideas as to the production of focal necroses in human typhoid fever. The idea that emboli of large cells in the liver capillaries produce anemic necroses there has never appealed to me because it would seem necessary to occlude all the capillaries completely in order to cut off any area from its blood supply. The chance of getting extremely early lesions ought to clear up the origin of the large cells also. Dr. Mallory regards them as endothelial cells, but the matter is still open to debate.

DR. HUNTOON: I should like to ask Dr. von Wedel if he has made any absorption tests with these organisms?

DR. VON WEDEL: So far we have not made any absorption tests with these organisms, as the agglutination tests were made in duplicate and the results agreed in all tests. While the absorption test no doubt is more delicate I do not think that it would have given us any clearer results than we were able to get with the agglutination tests. We shall have to complete the absorption tests to secure more light on the nature of the typhoid agglutination.

A CASE OF CHORDOMA OF THE SACRUM

F. C. WOOD, M.D.

Some years ago I demonstrated to the members of this Society the material removed from the sacral region of a woman of about thirty-five years of age. The clinical diagnosis in the case was myxosarcoma, but the peculiar vesicular cells lying in a homogeneous interstitial substance attracted my attention, and a careful study revealed the fact that the tumor was undoubtedly of the type known as chordoma.

These tumors arise from remnants of the chorda dorsalis, a structure which largely disappears before birth. Occasionally, however, remnants are persistent and undergo hyperplasia, resulting in the formation of small tumors which remain local and cause little or no disturbance or become malignant and invade the surrounding structures. The specimen which I show to-night is of the latter type.

This large jelly-like mass is a recurrence of the tumor removed from the sacral region four years ago, the removal of the mass requiring excision of a large part of the gluteus maximus muscle and the tissues of the right buttock. It is evidently of a low grade of malignancy, as the tumor cells tend to penetrate along the muscle bundles, and do not involve diffusely all the structures alike, though sufficient invasion of connective and fat tissue has occurred to warrant considering this growth as a malignant tumor.

Microscopical examination of the sections shows a typical gelatinous basement substance, in which lie large vesicular cells with relatively small nuclei. There is no question of confusion microscopically between this tumor and a myxosarcoma.

THE ATTITUDE OF UNDERTAKERS TOWARD THE PERFORMANCE OF AUTOPSIES

BY W. G. MACCALLUM

I thought it would be of considerable interest for this Society to know what happened yesterday at a meeting the Undertakers' Association at which Dr. Ewing, Dr. Janeway and I were invited to speak as to our point of view in regard to autopsies.

There were gathered together at this meeting a representative body of about seventy-five undertakers from this and adjoining states. They were evidently not entirely in sympathy with our interest in the performance of autopsies, although they were interested in having our point of view. We spoke in the tenor which you might expect—I need not give that in detail.

The more interesting part of the meeting was the discussion brought forward by the undertakers and I think we should know their point of view. With our various general statements about the matter of autopsies, they agreed perfectly, but they had the following grievances to air, concerned chiefly with autopsies performed in hospitals. Their grievances related not only to the doctors but also to executive officers of hospitals. They asked incidentally why we did not do autopsies on private cases but only did them on public patients in the hospitals. We easily cleared up this false impression.

1. They claimed that they were not treated courteously when they went to hospitals, though they asked for no particular favors, but merely for the death certificates and permission to take the body away; that they were kept waiting for hours, sometimes for the body and sometimes that the autopsy might be finished,—by a relatively slow pathologist. They thought that at any rate they should get the death certificates promptly.

2. That the death certificates were frequently made in such an obscure way that it was difficult to tell the cause of death. On this account they often experienced difficulty in having the certificates accepted by the health authorities.

3. That the services of the undertaker were often bargained for by a clerk at the front door of the hospitals, who would wait for the highest bidder and turn the funeral into his hands.

Those were the grievances as far as the executive side of the thing went. They also appreciated the fact that superintendents of hospitals are opposed to autopsies through fear or the wish not to be entangled in any kind of lawsuit.

They claimed that autopsies were done in the most brutal fashion; that they were done incompetently and not with the care that should be devoted to them. These complaints did not refer so much to external mutilations as to disturbances in the internal arrangement of the body which interfered with their embalming processes. They complained bitterly of the removal of the organs of the neck, which so interrupted the arterial system as to make it impossible for them to embalm the face. They also complained of the mode of removal of the skull cap and brain which often leaves a ridge across the forehead.

They welcomed the suggestion made by Dr. Ewing that the embalming be done before the autopsy. But under such conditions the pathologist could make nothing out of the gross appearance of the organs and no fine microscopic studies could be made. Furthermore, blood cultures would obviously be out of the question. The suggestion was made that we might embalm the head and neck for the undertaker or else call him in to do it before the autopsy was begun.

I have brought this matter up because it seems to me that we might meet the undertakers half way and follow their suggestions as far as possible, since they seem to have control of the whole situation. Dr. Janeway pointed out that, while the percentage of autopsies in German institutions ran as high as 95 to 97, in New York City it is only 15. It seems to me that we can improve this lamentable condition if we try.

DEMONSTRATION OF CULTURES OF THE VIRUS OF POLIOMYELITIS

SIMON FLEXNER, M.D., AND HIDEYO NOGUCHI, M.D.

The starting point of the cultivation of the virus of epidemic poliomyelitis was the successful cultivation of various spirochætæ by Noguchi. You are probably all familiar with the manner in which that cultivation was accomplished. Essentially, it consisted in the use of fresh tissue, suitable nutrient media, and the regulation of the amount of oxygen in the tubes. It was ascertained that the cultivation of the strictly parasitic tissue spirochætæ such as *Treponema pallidum* and *Treponema pertenue* required strictly anaerobic conditions, while the cultivation of the species that resides in the blood, among which are *Spirochæta recurrentis, Duttoni* and *gallinarum,* demands the presence of a minute amount of oxygen. Besides the fresh tissue the nutritive substance was chiefly ascitic fluid, but it was found that not every sample of ascitic fluid was suitable but that many samples were unsuitable. Hence, a careful selection of this fluid needed to be made.

As you know, many unsuccessful attempts have been made to cultivate the microorganism of poliomyelitis. On the basis of the successful cultivation of spirochætæ we undertook, in connection with the experimental work being conducted at the Rockefeller Institute on poliomyelitis, a cultivation of the virus of this disease. For this purpose Noguchi employed materials derived both from human beings and from monkeys suffering from epidemic poliomyelitis. Portions of the central nervous system, either in substance, or emulsions, or Berkefeld filtrates from the central nervous system, were employed for the cultivations, and not only were fresh tissues used but also tissues which had lain for many weeks or months in fifty per cent. glycerine. The cultural conditions provided consisted of sterile tissue, usually a fragment of the kidney of a rabbit, ascitic fluid and agar. The original cultivations were always obtained in a fluid medium and never on a solid medium. The fluid medium is overlaid with a layer of sterile paraffin oil to exclude oxygen. The growth in the fluid medium takes place slowly and in the first generation usually requires from seven to twelve days to become evident and produces no visible alteration of the medium. The growth begins in the lower end of the tube next the tissue. From this fluid culture a growth in solid medium is obtained by transfers into a medium consisting again of tissue, ascitic fluid and agar. The transfers from tube to tube are made at intervals of seven to twelve days and it may not be until several successive transfers to a solid medium have been made that a growth is secured. This growth consists of discrete minute colonies beginning in the bottom of the tube and rising in but not reaching the surface of the medium. Stained preparations of the fluid and then of the solid growths show the microorganism to consist of minute dots occurring singly, in pairs, masses, and even in chains. The microorganism is stainable by Giemsa's and also by Gram's stain. The latter acts irregularly and sometimes is readily decolorized.

The microorganism measures about 0.15 to 0.3 μ in diameter. In form it is usually globular but sometimes ovoid or laterally flattened. It will pass the pores of Berkefeld filters *V* and *N*. It is immobile. The microorganism is found in the tissues of man and monkeys suffering from poliomyelitis, both in film preparations and in sections. Since others may be sufficiently interested to undertake the cultivation of this microorganism it is desirable to emphasize the fact that to obtain the initial growth is difficult and that its demonstration in tissues is also difficult and the usual staining methods fail to bring it into view.

The microorganism is very simple in its structure and therefore the determination of its etiological relationship must be brought by animal inoculation. Without success in this field it would hardly be possible to attach the microorganism etiologically to epidemic poliomyelitis. We have succeeded in producing poliomyelitis in a number of monkeys by inoculating the cultures. The successful inoculations have been conducted both with cultures derived from human tissues and from monkey tissues. It has been found less difficult to produce the infection up to the present with cultures less far removed than with those further removed from the original materials out of which they have been developed; which probably means, as was predictable, that the cause of poliomyelitis is a highly parasitized microorganism. And we all know how difficult it is to maintain such parasitized organisms in a state of virulence under saprophytic conditions of growth.

The incubation period after inoculation of the culture is similar to that observed in inoculating directly an emulsion of monkey spinal cord and may be as brief as four or five days or as great as two or three weeks. The symptoms developed are characteristic of poliomyelitis in the monkey and the lesions in the central nervous system are indistinguishable from those occurring in human beings or produced in monkeys with infected human or monkey tissue. After successful inoculation, except at the local point of introduction of the culture and even not always there, it is as difficult to demonstrate in tissues the microorganism as is the case in the disease produced by emulsions of infected tissues. The tissues of monkeys which have been inoculated successfully can be transferred directly to other monkeys, as can infected monkey tissues ordinarily derived, and can be preserved in glycerine in an active state. In other words, there is accurate relationship between the experimental disease as produced by the cultures and the experimental disease as produced by inoculating infected human or monkey tissues. Indeed, not only are the symptoms and the lesions identical, but we have also observed instances in which a polioencephalitis has been produced by the cultures either alone or along with a poliomyelitis. In the human affection as well as in the experimental disease as ordinarily produced in the monkey the lesions are, as we now know, not limited either to the anterior horns or even to the gray matter of the spinal cord but affect also the white matter and, as has also been observed by us, with great constancy the intervertebral ganglia. This same distribution of lesions has been repeatedly observed in monkeys inoculated with cultures, so that in minute details correspondence is accurate.

In our original publication we withheld decision as to whether there might be an association of microorganisms in the cultures, namely, along with the visible organism the growth of a second and invisible microorganism. We see no way by which this point can be readily excluded except by that of indefinite dilution which we think must have been accomplished in the later generations.

Discussion:

Dr. Wadsworth: This seems to be an exceptionally complete demonstration of the etiological relationship of this organism to poliomyelitis except in the one point which Dr. Flexner has emphasized so clearly, namely, the possibility of the association of an invisible virus with the organism. I should like to ask Dr. Noguchi whether he has obtained any growths on solid media from which transfers might be made.

Dr. MacCallum: As I understand it Dr. Flexner and Dr. Noguchi do not make any attempt at classification of this organism. I here get the impression that this may be the first organism of a new group to be rendered visible and we shall await with interest the decision as to whether it is an animal or plant.

Dr. Noguchi: It seems still too early to form a definite opinion as to the nature of this organism, but we may get a better understanding when similar organisms are obtained in culture one after another. It is interesting to note that the cultural conditions required by this organism are similar to those necessary for the spirochætes.

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DR. A. B. WADSWORTH, *President*

A CASE OF OVARIAN GESTATION

M. CATURANI

Among several specimens of ectopic pregnancy upon which I have been working at the Pathological Laboratory of Cornell University, one specimen, on microscopical examination, proved to be an ovarian pregnancy. Through the courtesy of Dr. Ewing I have the pleasure to submit this specimen to your attention, together with a few remarks on the history of the case.

Case History: Patient 33 years old; has had three children; last child five years old; has had five abortions; history of gonorrhea and syphilis. In June, 1909, I operated upon her for a right tubal abortion; tube and ovary on the right side were removed. In January, 1912, after a few days delay of the menstrual period, the patient came to me with all the symptoms of an ectopic pregnancy on the left side. At the operation, fifteen days after the initial attack, the abdomen was found filled with clotted and fluid blood. The clots were mostly in the region of the left adnexa; the fimbriated end of the fallopian tube was patulous; the ovary on the distal pole was covered by clots and was damaged. The left tube was removed; the ovary was not removed but resected, on account of the absence of the right ovary, which had been removed at the previous operation. The case at the time was considered one of tubal abortion, but failed to show any evidence of ovum in the tube.

The gross specimen shows the fimbriated extremity of the fallopian tube patulous. The resected ovary is attached by a band of tissue to the mesosalpinx. This part of the ovary measures 3 cm. in width and 2 cm. in thickness. A section for microscopical examination was taken from the inner side of the specimen. This section shows a circular, whitish band of tissue surrounding a dark central area. Microscopical examination of the various sections shows a large area of blood and fibrin, surrounded by ovarian tissue, and in the hemorrhagic areas are scattered chorionic villi. Villi are present in all parts of the central area, beginning from the inner edge of a distinct corpus luteum. In some sections chorionic villi are seen among the columns of lutein cells. In places the decidual reaction is very slight, but there is a group of distinct Langhans cells in the cortex of the ovary.

The integrity of the tube and the presence of fetal elements closely associated with the corpus luteum, surrounded by ovarian tissue, constitute the essential features of the specimen.

LUNG FROM A CASE OF ARTIFICIAL PNEUMOTHORAX

LEO KESSEL, M.D.

The compression of a tuberculous lung was first noted as having beneficial effect by Carson, a physiologist of Liverpool, in 1822; and ten years later Houghton reported a case of pulmonary tuberculosis complicated by pneumothorax. Following this there occurred in the literature from time to time occasional references to cases in which beneficial results had been exerted by spontaneous pneumothorax, following which fibrosis had occurred. In 1888 Forlanini advocated the introduction of nitrogen gas for the compression of the lung. This idea was suggested at the Congress in Rome, but was not generally received with enthuiasm, although fifteen years later several cases were reported as having been benefited, and the idea was more widely received About this time Murphy of Chicago reported five cases. In Germany also work was carried on along this line by Brauer and Spengler, and in this country it has been done more recently by Floyd of Boston. At the present time this work is being admirably carried on by Robinson, and the Bedford Sanitarium is largely interested in this method.

Various methods have been suggested in connection with the introduction of nitrogen gas, such as the puncture method by Forlanini and the incision method by Brauer. The former has been used by us. I have had the opportunity of studying a large number of cases, treated at the Bedford Sanitarium by the method of artificial pneumothorax. The case presented is of a patient there.

This woman entered the sanitarium in 1913, with a history of cough, profuse expectoration for two and a half years, and in recent months there had been chills, fever, night sweats, emaciation, progressive weakness, and cyanosis. The temperature was 104 . Chills, sweats and an absolutely intractable cough persisted. There was a cavity in the upper lobe of the lung on the right side; she also suffered from mitral insufficiency. The patient was classified as a "third stage" case, and the clinical course confirmed this. The patient happened to be placed in a ward where there were a number of other cases who were undergoing the artificial pneumothorax treatment. She begged very hard to be allowed the chance of this treatment. We were very unwilling to grant this request as there were many contra-indications. There were bilateral lesions and the patient was practically moribund, with marked cyanosis, and her cough had failed to yield to any form of treatment. Finally it was considered possible that we might collapse the lung cavity and the treatment was determined upon. There was very little difficulty in entering the left chest and ten inflations of nitrogen gas were administered, not more than 500 c.c. being given at any one time, and usually not more than 250 c.c. at a dose. The points of entrance were twice in the fourth space in mid-axillary line, four times in the seventh space in the posterior axillary line and four times in the ninth space in line with the vertebral border of the scapula.

From the first the patient did remarkably well. The temperature came to normal; the intractable cough subsided; expectoration diminished; the patient was able to sleep and was much better in every way; she was even able to get out of bed. This continued for ten weeks after the establishment of the pneumothorax, when, rather suddenly, her condition changed for the worse: dyspnœa and cyanosis returned and all the symptoms were aggravated. She lost ground rapidly and died in ten days. Death was undoubtedly due to cardiac decompensation and acute dilatation on the right side of the heart.

A point of interest was that, in spite of the complete collapse of the left lung, at no time was there absence of breath sounds or rales. This shows clearly the misleading features of physical diagnosis and that it is essential to this work that a good X-ray should be obtained. The physical examination did not show complete pneumothorax at death. There was an extensive obliteration of the entire right pleural cavity; the left was practically free. The X-ray plates show the condition of the lung, first, before pneumothorax, second, after pneumothorax. In the second the adhesions are very distinct from the pleura up to the second space, and behind to the fourth space. The left pleural cavity appears practically free. At death there were three ounces of clear fluid in the pleural cavity. The lung was found to be absolutely compressed against the heart and the posterior mediastinum. The pleura was smooth and thickened. There was a marked mitral insufficiency and dilatation of the right ventricle. There was a chronic parenchymatous nephritis and a tuberculosis of the ileocæcal junction.

The X-ray picture of the lung shows the extreme compression which can be exerted by this method. An adherent pericardium is shown. The adhesions are very tough and no amount of pressure would influence any pocket formed in the pleuræ, made by adhesions of such density. Pressure, on the other hand, in such a case, is very much more likely to effect a rupture of the lung and these instances occur much more frequently than they should. Another point shown is the way in which adhesions can remain persistent between the thoracic wall and the pleura over a cavity. Gas is introduced, but the failure of clinical improvement is accounted for by the non-collapse of the cavity.

The experience of two men, Droscher and Gretz, shows that an artificial pneumothorax sometimes induces a well marked fibrosis. Kaufman and Bruns, in their experiments on dogs and rabbits, show that atelectasis and fibrosis may be induced.

Discussion:

Dr. Wadsworth: It is interesting to see the pictures of this case and, at the same time, the autopsy material. Has Dr. Kessel made any study of the tubercle bacilli in the different lesions, to determine their number and distribution in the lesions of the treated lung, as compared with the other lung?

Dr. Pappenheimer: Do the lesions in the other lung show the same organization?

Dr. Mannheimer: I have seen, in the case of a big, healthy dog, the lung, which had been compressed by repeated insufflation of nitrogen gas into the pleural cavity. This had been kept up for six months, and then, after an interval of another six months, during which the animal gained in weight and was perfectly well, it was killed, and the lung was found in the following condition. It was one fourth to one third of its original volume, felt tough, and there was still a bubble of gas in the sac. Microscopically there was no complete atelectasis, but the alveolar walls were approximated and thickened and included infiltrations of desquamated epithelia and red cells. The connective tissue around vessels and bronchi was markedly increased, as well as that of the pleura. That means the healthy lung of a strong animal had been so affected in structure and function by a pneumothorax, that after a prolonged rest it could not be re-expanded. Similar conditions have been reported on human cases, *e. g.*, a connective tissue overgrowth, not only in the tuberculous areas of the lung where we want it, but also in the healthy areas where it is not wanted. We should therefore not accept the indications set by some enthusiasts at the last International Congress, namely to perform artificial pneumothorax in incipient cases.

Dr. Kessel: In answer to Dr. Wadsworth's question, it may be stated that in one of Droscher's cases, in the collapsed area there was no tendency to growth of new tubercle bacilli. It is exceptional to find evidences of fresh bacilli in a collapsed lung. In reply to Dr. Pappenheimer, there was some activity of the tuberculous process in the right apex, but no evidence of organization at all.

TWO SPECIMENS OF SARCOMA OF THE INTESTINE

A. B. EISENBREY

The specimens to be presented are from cases operated upon at St. Luke's Hospital during the summer of 1913. Two cases of sarcoma of the intestine, one a lymphosarcoma of the ileocaecal valve, the other a spindle cell sarcoma of the cecum.

The lymphosarcoma was found in a girl seven years of age, sent in with a diagnosis of acute appendicitis. Her family and previous history were negative. Examination showed symptoms indicating acute appendicitis. Blood count showed a leucocytosis of 10,500, polynuclears 80 per cent.

At operation the appendix was found to be moderately inflamed and a tumor was palpated at the ileocæcal junction. The lower part of the cæcum and about 4 cm. of the ileum were resected. The tumor was a firm, rounded mass measuring 3 × 2 cm. arising apparently in the posterior portion of the ileocæcal valve. Surface appeared necrotic. On section the tumor presented a smooth, homogeneous, glistening appearance, color pinkish yellow. Necrosis extended only 1 mm. beneath the surface. On the inner aspect of the cæcum there are two enlarged, rather firm lymph nodes. Similar nodes were found in the mesocolon.

Microscopical examination shows delicate, loose connective tissue reticulum with a few thicker trabeculæ. The spaces so formed are closely packed with small round cells and deeply staining nuclei. The tumor is actively invading the surrounding tissue, including the interstitial tissue of the mucosa. It is only slightly vascular. Sections of the glands show no neoplasm. The examination of the appendix shows an inflammatory process confined to the mucosa.

The patient developed a fecal fistula and later returned to the hospital. On her entrance she had a leucocytosis of 53,000, polynuclears 70 per cent., lymphocytes 93 per cent., red blood cells 4,453,000, hemoglobin 50 per cent. When free drainage was established leucocytosis fell to 12,000, polynuclears 13 per cent., lymphocytes 87 per cent. The polynuclear-lymphocytic ratio showed little change until her death six weeks after the operation. No autopsy was obtainable. Her anæmia was progressive and at the time of death red blood cells were reduced to 2,300,000, hemoglobin 25 per cent.

The spindle-cell sarcoma was from a married woman 52 years of age. She entered the hospital complaining of a pain in the bowels and weakness. Four months previously she had had headaches, chills, fever and was constipated and in the intervening time had lost twenty four pounds in weight. Her last menstrual period occurred three years previously. She had had fourteen children and two miscarriages. Family history was irrelavent. Physical examination showed an abdominal tumor about 10 cm. in diameter in the lower left quadrant, which was hard, not tender, and but slightly movable. At operation the tumor was found to lie between layers of the mesosigmoid, and was adherent in the left iliac region. On removing it a large cavity containing fecal matter was opened.

The tumor measures 12 × 6 × 7 cm., and involves the wall of the cæcum, 20 cm. of which was removed with it. The cavity in the main tumor communicates with the lumen of the gut through two ulcerated areas about 1 cm. in diameter. On section the tumor has a homogeneous, "meaty" appearance, and there are enlarged lymph nodes beneath the peritoneum adjacent to it.

The tumor is made up of cells of the small spindle type and actively invades the wall of the gut. There is a marked suppurative process and much necrosis in the main tumor mass. The patient made a slow recovery and left the hospital five weeks after the operation.

NEURO-FIBRO-SARCOMA OF ARM

A. B. EISENBREY

The third specimen is from the left arm of a young man, nineteen years of age. He gave a history of having noticed, four years previously, a lump the size of a hickory nut at about the middle of the inner aspect. This increased slowly in size and has never been inflamed, painful or sensitive. He had no recollection of trauma and denied lues. Family history was negative.

At operation a fusiform tumor measuring 21 × 5 × 4 cm. was found lying in the sheath of the brachial vessels and nerves. No enlarged glands could be palpated.

The tumor is well encapsulated; a rather large nerve trunk runs into the apex of the tumor and its fibres fan out over the surface. From the lower extremity there extends a short nerve trunk which quickly forms three distinct trunks. On section, tissue is firm and fibrous. There are several areas of softening, numerous small spaces filled with clear yellowish fluid and a cystic cavity containing chocolate colored fluid.

Microscopic examination shows a general fibrous structure similar to that usually described in neurofibromata. In addition there are areas, particularly at the upper extremity, which show marked variation in the shape and size of the cells, many of them being of large size, irregular in outline and there are some multinucleated cells. A few cells are found which are suggestive of neural elements but up to the present we have been unable to demonstrate their nature by staining reaction. A tentative diagnosis of neurofibrosarcoma is made and I shall be glad to submit the material to the Committee on Microscopy.

Discussion:

Dr. Libman: The first case was of interest to me because the clinical picture was that of acute appendicitis. A number of years ago I published five cases of lymphosarcoma of the intestine and drew attention to the fact that certain cases have the clinical picture of acute or subacute appendicitis. The resemblance to appendicitis in some of these cases is probably due to the interference of the action of the ileocæcal valve. If there is obstruction to the valve or if there is edema and inflammation of the valve, one can get very marked symptoms analogous to what one finds in acute appendicitis. Mackenzie has pointed out that a number of symptoms are probably due to the hyperperistalsis of the small intestine during the attack. Several years ago I saw a case of an ordinary ulcer of the cæcum without any thickening of the wall but with inflammation of the ileocæcal valve and marked edema of the valve. In this case the patient suffered from symptoms like those one sees in cases of appendicitis with secondary obstruction of the bowels.

The lymphosarcomata of the intestine generally become quite large and extensive before they produce symptoms. The spindle-cell sarcoma can produce marked symptoms even if they are quite small. Besides typical cases of lymphosarcoma of the intestinal tract and lymph nodes, and besides the pseudoleukemia cases and cases of Hodgkin's disease, there are atypical forms of disease which are difficult to classify. At times the lymphosarcoma is described as starting in the intestine, when it really starts in the mesenteric nodes. The cases that are found in the mesenteric nodes and involve the intestine secondarily can sometimes be distinguished by the fact that they produce peculiar traction diverticula in the intestine.

Dr. Ewing: Lymphosarcoma of the intestine is a subject of very great interest both on account of its frequency and because of the obscurity of its origin.

The present case illustrates that form of the disease which is localized and locally aggressive. It is apparently a very early stage of the condition in three cases of acute lymphosarcoma clinically resembling appendicitis presented some years ago by Dr. Libman. In a second group of cases the lesion is very extensive, affecting much of the small intestine and colon, with local tumors and ulcers. Such cases are described by Stoerck (*Wien. kl. Woch.*, 1894).

In a third group the stomach is the chief seat of the lesion and many retroperitoneal nodes are involved. This lesion is locally destructive and early invades the nodes with the production of large tumors. A primary intestinal lesion is often missing and the disease, while probably of gastro-intestinal origin, runs its course in the abdominal lymph nodes.

Finally, there is a very diffuse lymphomatosis of the entire gastro-intestinal tract from cardia to anus, anatomically lymphadenoma, not locally aggressive, but systemic, with tumors of cervical, thoracic, and abdominal nodes. Some years ago I presented such a case to this Society.

All of these processes seem to be different manifestations of one and the same disease.

In various cases examined in our laboratory, we have found evidence suggesting that the disease is a modified form of tuberculosis or some closely related infection.

EXPERIMENTAL NEPHRITIS CAUSED BY REPEATED PROTEIN INTOXICATION

W. T. LONGCOPE

I have brought lantern slides illustrating experiments to be described. These experiments were suggested by a study of certain cases of nephritis observed at the Presbyterian Hospital, in some of which there was a clinical picture which reminded one of serum disease, as described by von Pirquet. Though careful studies have been made of the changes of the organs of animals dying of acute anaphylactic shock, little attention has been paid to the study of repeated anaphylactic shock experimentally. It has, therefore, seemed important to investigate this subject, in order to determine whether, under such conditions, anatomical lesions might appear in animals. For this purpose several species were employed, namely,—guinea-pigs, cats, rabbits and dogs. These animals were sensitized, either to horse serum or egg albumin, and then after a period of three weeks, injected intravenously or intraperitoneally at varying intervals of time with these foreign proteins.

Though many animals became refractory and did not show the symptoms of anaphylactic shock after six or eight inoculations, still it was possible in many instances to produce repeated shocks over periods of weeks and even months. Animals subjected to single sensitizing doses showed no changes in the various organs, but those subjected to repeated intoxicating doses presented changes in the peritoneum, when the inoculation had been intraperitoneally, or, under any circumstances, in the liver, heart muscle and kidney. Changes occurred in the kidney in over 80 per cent. of the 66 animals employed, and were most constantly observed in rabbits and guinea-pigs. Earliest changes showed themselves as necrosis of the epithelial structures, and were observed in the cells of the convoluted tubules and the collecting tubules. Following such necrosis, there was an extensive infiltration of small round cells which produced a picture in many instances of an acute interstitial nephritis. Later there occurred an increase in the connective tissue which was most marked in the intermediate zone, extending from this region into the cortex. In a considerable number of instances there were also lesions in the glomeruli. Earliest changes seen consisted in swelling of the capillary endothelium, with karyorrhexis of the nuclei and infiltration of the small mononuclear cells. The capsular tufts contained almost no red blood cells. In cats and guinea-pigs there were occasional isolated glomeruli which showed well marked proliferation of the cells lining the capsules, which led to crescentic formations. In the later stages of the process, glomeruli were often completely changed by proliferation of the capsular cells, and atrophy of the glomerulus.

Realizing that spontaneous lesions in the kidney are not uncommon in all laboratory animals, control experiments were instituted to exclude the possibility of spontaneous lesions as a source of error. Three dogs were sensitized to egg white and horse serum and submitted to operation; a portion of one kidney was dissected and in each instance the kidney proved normal. After an appropriate period, intoxicating doses of egg white and horse serum were injected intravenously. In one dog after four inoculations, another after six, and a third after ten, well marked changes, similar to those already described, were found at autopsy in the kidneys.

These observations in the high percentage of positive results seemed to exclude as nearly as possible spontaneous lesions as a cause of the anatomical lesions described. As to whether the lesions are dependent merely upon a toxic action of these proteins themselves, or whether it is associated actually with the anaphylactic intoxication, has not yet been definitely determined, though as far as my observations go, it does not seem possible to produce such lesions by single injections of large quantities of the foreign protein used. This would point to the assumption that the lesions areare in some way dependent actually upon the development of the repeated anaphylactic intoxication to which they have been submitted.

Discussion:

Dr. Wadsworth: What quantities were inoculated?

Dr. Longcope: Guinea-pigs received very small quantities, as 1 c.c. of egg albumin or horse serum; rabbits had three doses of 2 or 3 c.c. before they became sensitive; dogs sometimes had intravenous, sometimes subcutaneous doses of 3 or 5 c.c.; cats received small quantities,—for intoxicating doses 1 to 1.5 c.c. was sufficient; for dogs 3 to 10 c.c. Very small quantities were sufficient to produce symptoms in sensitized cats.

Dr. Butterfield: The question arises as to where can the line be drawn as to what is nephritis and what is not? Are we to use the term for various pathological alterations of the kidney, or are we to limit the term to a diseased state, characterized by certain functional disturbances, as described by Bright?

Dr. Baehr: It is interesting that in the experimental nephropathy induced by Dr. Longcope in his rabbits, the most marked lesion appears in the zone between cortex and medulla. As Suzuki recently demonstrated, the so-called "convoluted tubule of the first order" can be arbitrarily subdivided into four divisions, according to the intensity with which the epithelium takes the vital stain. The first and second divisions are very convoluted and lie in the immediate neighborhood of the glomerulus belonging to that tubular system; the third runs a spiral course and lies chiefly in the zone between cortex and medulla; the fourth runs more or less deeply into the medulla before it changes into the descending loop of Henle. It seems to me that it is the third portion of the "convoluted tubule of the first order," called by some the canalicula spiraliforma of Schachowa, which has been picked out by the poison in Dr. Longcope's experiments. An accurate localization of the damage is extremely important, for, as I have frequently demonstrated, for the human kidney (*Ziegler's Beitrage*, LVI, p. 1, 1913), the various divisions of the "convoluted tubule of the first order" probably possess specific functions. Only through such an accurate localization of the damage will a proper interpretation of the physiology of the different portions of the tubular labyrinth of the kidney be possible.

One further point to which I desire to call attention concerns the interesting glomerular changes observed by Dr. Longcope. If I understand him accurately, they appeared rather late in the process, several weeks after the tubular damage. This fact, together with the character of the morphological changes in the glomeruli, leads me to believe that the lesion may not be due directly to the action of the poison. It seems more likely that it is merely a secondary lesion subsequent to the damage lower down in the tubular system.

Dr. Weil: We have ample evidence that foreign protein produces widespread degeneration in various organs. Gay and Southard have found that a single anaphylactic shock produces acute lesions. In a dog after a second injection I have obtained very striking lesions. The lesions are extreme; there is tremendous congestion of various organs. After a second injection in guinea-pigs we have found cloudy swelling of the cells of the kidney and liver. I should like to ask if any involvement of the nervous system has been observed? If rabbits are repeatedly injected with horse serum sometimes one gets paralysis of lower extremities, and an appearance like torticollis. There must be lesions in the central nervous system to explain these conditions.

Dr. Wadsworth: The experimental study of the lesions of anaphylaxis, such as Dr. Longcope has brought forward, is a comparatively new and important field of research. It is of considerable interest to determine the nature of the action of the poison on the cells of the kidney: whether the lesions are due to the action of poison derived from the breaking down of the foreign protein used in bringing about the anaphylactic reaction, or to the products of disturbances in the metabolism in the tissues and organs generally, resulting from the anaphylactic reaction. Just how the anaphylactic reaction is brought about is obscure, whether it is due to poisons derived from the foreign protein introduced, or to more subtle changes in the body tissues resulting from the presence of the foreign protein is not yet clear. Has Dr. Longcope, from the results of his experiments, been able to shed any light on these phases of the problem?

Dr. Longcope: Replying to Dr. Butterfield, I have carefully avoided making any mention of clinical significance of this experiment. The lesions in the kidney do not simulate ordinary lesions of clinical nephritis. Lesions in the liver are not like cirrhosis of the liver, and lesions of the heart are not altogether like those in man. That is entirely beside the point. It is merely a study of repeated protein intoxication. In reply to Dr. Baehr, the glomerular lesions are perhaps secondary in some instances, but where no interstitial lesions were seen about the tubules it seems possible that there might be primary injury to the glomerular vessels themselves. In reply to Dr. Weil, Richet has studied lesions in the central nervous system, and has not reported any changes. Chronic changes are only produced by repeated injection of foreign protein; a few injections do not produce chronic changes.

THE HISTOLOGICAL FINDINGS IN THREE CASES OF ADAMS-STOKES SYNDROME

ADELE OPPENHEIMER, M.A., AND B. S. OPPENHEIMER, M.D.

INTRODUCTION

Although a few years ago the problem of permanent heart-block seemed almost settled by the discovery of lesions in the conducting system, it is now apparent that the solution of the problem is not so simple as it appeared at that time. There are cases on record in which heart-block was proved beyond a doubt, but still no lesions in the hearts adequately explaining the failure of the conduction of impulses from auricle to ventricle were to be found.

The three cases to be presented this evening show three very different histological conditions in the conducting system; and it is somewhat unfortunate that the clinical data do not give us such definite information of the mechanism of the heart as can be positively correlated with these post-mortem findings.

Three hearts from patients with a clinical history suggesting heart-block were kindly entrusted to us for microscopic examination; two by Dr. MacCallum and one by Dr. Bishop.

HEART I

Case Report.—The patient, a male aged 75 years, is described by Dr. Bishop as having had a slow pulse, vertiginous and syncopal attacks and rapid venous pulse. A polygraphic tracing was published with the clinical history in the *American Journal of Medical Sciences* for 1910.

Macroscopic Examination.—The post-mortem examination of the heart was made by Dr. Larkin, who has already demonstrated the heart grossly at one of the meetings of this society. It showed a calcareous nodule directly in the normal pathway of the bundle of His.

The cusps of the aortic valve were extremely thickened, the wall of the aorta itself showed patches of atheroma; similarly the coronary arteries both right and left presented a very marked arteriosclerosis.

Microscopic Examination. The auriculo-nodal connections, i. e. the connections between auricles and node of Tawara, are attenuated but not actually interrupted. The nodal artery shows increase of connective tissue in the intima and media; indeed there is sclerosis of the vesels throughout the whole of all the sections. The node of Tawara and the main stem of the bundle of His are continuous. The stem is characterized by a greater vascularity, more marked cellular infiltration and the presence of more connective tissue than is normally the case. The stem progresses as usual through the membranous septum and comes to lie under the endocardium of the left ventricle when abruptly at its forward end it abuts upon a calcific necrotic sphere, there bifurcating to form what were judged to be the stumps of the right and left branches. This calcified sphere which lies directly across the stem at its bifurcation stretches from the endocardium of the left ventricle almost to the endocardium of the right.

There are other calcific nodules and one of these indents the stem at its narrowest part and apparently compromises it.

Summary.—The upper ends of both limbs almost wholly and the body of the stem for about 1 mm. of its length have been demolished by this necrotic calcifying process; so that there is in this case an actual break in the course of the A–V conducting system.

HEART II

Case Report.—The heart was forwarded to Dr. L. F. Barker at Johns Hopkins by Dr. D. A. Campbell, professor of medicine, Halifax, Nova Scotia, together with notes.

Dr. Campbell first saw J. C., a retired mariner, in the summer of 1905. He was then in his ninety-fifth year. During the summer of 1906 he was ill the greater part of the time, but never wholly confined to bed. The most pronounced symptoms were: mental depression, cyanosis, bradycardia; the pulse rate varied from ten to forty without any irregularity. There were slight seizures resembling petit mal occurring very frequently some days, and Cheyne-Stokes respiration especially at night. He kept fairly well for a period of ten months, taking belladonna the greater part of the time.

Early in August, 1907, the symptoms returned and gradually increased in severity, causing his death a month later. The bradycardia was not so marked as before, thirty being the lowest pulse rate noted, and the epileptiform seizures were infrequent. The more prominent terminal symptoms were: marked cyanosis, Cheyne-Stokes breathing, and total disappearance of the radial pulse and cardiac impulse for periods varying from thirty to sixty seconds in duration.

Microscopic Examination.—S–A Node Region.—The sinuauricular node is sclerotic. Even allowing for individual variation within normal limits, the increase of connective tissue in this case is so marked throughout the entire extent of the node that it may be safely stated to be abnormal. No such fibrosis is present in the musculature immediately about the node or in the other portions of the right auricle examined. In the main the nodal fibres themselves may be described as lacking in cross striations, an appearance evidently associated with sclerosis of the node. The nodal artery, present in this heart, shows sclerosis of the media and a slight endarteritis. Some of the smaller branches too show these changes, as do also the artery on its way to the node and some of the vessels among the ordinary cardiac musculature; occasionally a vessel presents obliterating arteritis.

Here and there in the pectinate and other ordinary cardiac musculature as well as in the specialized musculature of the Keith-Flack node, a very few cells which resemble lymphocytes are seen. Larger collections of them appear in the epicardium and subepicardium; the largest at the level of the pace-making apparatus. Sometimes they are found about the subepicardial nervous elements in the neighborhood of the node.

In general the effects of the pathological changes in the node of Keith and Flack are not yet established, and we do not venture upon judging the significance of the sclerosis of the structure in this case.

Region of the A–V System.—The connections between the auricular musculature and the node of Tawara are scanty. The node in the sections is an irregular semi-circle, the diameter of which lies along the central fibrous body, and the circumference of which is almost entirely surrounded by fat tissue, thus practically separating the nodal from the auricular musculature.

Cross sections of the artery in the specialized musculature present sclerosis of the intima and media. Some of the very small vessels have a marked diminution of their lumina; but it is impossible to say which of the two inner coats is involved. There is intimal and mesial sclerosis of the arteries in the interauricular and interventricular septa, and of the coronary artery (with atheroma). There is calcific deposit in the wall of the aorta not only near the opening of the right coronary but even nearer to the A–V system than that.

Though the stem is long and slender, there is nowhere a break in its continuity.

Summary

While it cannot be affirmed that the slightness of the auriculo-nodal junction is the causative factor in this case of Adams-Stokes disease, yet it is of importance in the light of the fact that Armstrong and Mönckeberg reported an exquisite case of lymphangioendothelioma of the A–V node in which it was judged that the break between auricular and nodal musculature was the cause of the heart-block.

The condition of the blood vessels in the aged patient may have been an accessory factor.

We should expect to find some cellular infiltration in arteriosclerotic hearts. However we do not think that there is any relation between the increase of cells in the interstitial tissue of the S–A node and of some of the ordinary auricular musculature and the clinical symptoms.

In brief, the most obvious explanation of the symptoms in this case is the auriculo-nodal break.

HEART III

Clinical History.—The heart was received from Dr. W. G. MacCallum who performed the autopsy and gave us the clinical notes furnished him by Dr. M. G. Porter.

The patient was a white man aged 50. There was no history of syphilis; he had not used alcohol to excess. He had always been strong and healthy until 18 months before exitus when he had suffered from several attacks of syncope within a few days. Six months before his death he had a similar seizure and since that time he had frequently complained of giddiness on rising and of shortness of breath.

Three days before his death he was seized, after violent exertion, with severe pain in his precordium, radiating down both arms. Subsequently he had repeated attacks of syncope and several epileptiform convulsions with attacks of bradycardia between which the pulse rhythm would be normal. The pulse rate ranged between 110 and 25 beats per minute. On the day previous to his death a distinct pericardial friction sound was heard on two occasions ("Pericarditis Epistenocardia"—Sternberg). The patient died in one of his seizures.

Autopsy.—To quote Dr. MacCallum: "The pericardium was found to be filled with blood which was partly clotted; in places the clot was fully 1 cm. thick. There was a ragged hole in the anterior wall of the left ventricle about 1 cm. to the left of the vein that corresponds to the interventricular septum. Some blood clot was adherent to and extended into this ragged opening. The interventricular septum throughout its entire extent was opaque, of a grayish ochre color and was evidently necrotic. This appearance extended to within 1 cm. of the pulmonary orifice where it was sharply marked off from adjacent normal heart wall. The wall of the left ventricle was greatly hypertrophied, but its tissue was not abnormal.

"The trabeculæ were opaque and frequently broken through. In the angle between the wall of the ventricle and the septum was a hole, through which a probe was passed until it extended through the opening on the anterior surface of the heart. Both coronaries were markedly sclerotic. The anterior descending branch of the left coronary artery was completely occluded by a thrombus 3.5 cm. from its orifice. The sequence of events has evidently been: coronary sclerosis, thrombosis of the left descending branch, infarction of the interventricular septum, rupture of the heart wall, hæmopericardium."

Grossly then the heart presented infarction of the interventricular septum including the neighborhood of the main stem of the bundle of His and its branches.

Region of the S–A Node.—Unlike the portions from the interventricular septum, the block of tissue in the region of the S–A node presents a comparatively slight cellular infiltration. The round cells (only a few polynuclears are to be seen) are present in the epicardium and subepicardium, to a less extent in the nodal tissue, and to still less an extent in the ordinary myocardium. The vessels both of the specialized and typical musculature present sclerosis of the two inner coats.

It does not seem possible that the very slight cellular infiltration of the pace-making system in this case could account for any decided influence on the functions of the heart.

Region of the Upper A-V System.—A very few infiltrating cells—for the most part lymphocytic, rarely polymorphonuclear—are seen in the conducting system. Close at hand, at the basal end of the ventricular septum in an infected infarct with pus cells in the capillaries and adjoining tissues. There are pus cells elsewhere (everywhere round cells exceed the polynuclears in numbers), namely in the epicardium, subepicardial fat, auricular musculature, endocardium, tricuspid and mitral and aortic valves, but the most pronounced lesion in this block of tissue is situated in the typical ventricular musculature *near* the junctional tissue. Here the musculature has taken an unusual brown tint and many of the muscle nuclei seem to have disappeared. Indeed some of the muscle fibres themselves have apparently disappeared in their entirety and have been replaced by connective tissue. The remaining muscle fibres are separated by columns and masses of pus cells and are shrunken. Practically all the vessels present intimal and medial sclerosis.

Region of Terminal Arborizations.—There is in this adjoining block of tissue, in the ordinary ventricular musculature a continuation of the same lesion (an infected infarct) as has just been described. In that proximal portion of the left limb of the A-V system which was marked grossly with India ink there is no cellular infiltration, but ventral of this region there is some, where it is not unlikely that the left branch is involved. As to the distal terminal arborizations of the A-V system, there is some cellular infiltration of the ventricular musculature including that of the apex; on the other hand there is no such infiltration of the posterior papillary muscle. In other words there is no significant infiltration in the region of the branches on the septum or in the region of the terminations of the A-V system in the posterior papillary muscle and neighboring wall.

Summary.—In short, an almost negligible number of lymphocytes and a still lesser number of polymorphonuclear cells have invaded the auriculo-ventricular system, including the regions of its arborizations, and that is all; therefore from an anatomical standpoint the conducting mechanism is in a condition to functionate, but from a physiological viewpoint the sclerosis of its vessels and the presence of even a few pus cells in the junctional tissue and finally the neighboring infected infarction suggest an inefficiency of circulation and an abnormal condition sufficient to account for heart-block.

SUMMARY

(1) The hearts of three patients with Adams-Stokes syndrome presented, the first a calcific mass in the region of the A–V bundle, the second no such *gross* lesion in the pathway of the junctional tissue to account for the symptoms, the third infarction of the interventricular septum.

(2) The first case is reported to have denied a syphilitic history, in the second case the past history is not given, and the third case is said to have had no lues.

(3) In all three of these aged patients the sclerosis of the coronary arteries and its intermuscular branches suggest that an inadequate circulation played a part in the production of the symptoms of Adams-Stokes syndrome.

(4) As expected in arteriosclerotic hearts, some increase of the cells in the interstitial tissue was observed, but we do not know its significance in these cases.

(5) If these are three cases of heart block as is suggested by the clinical histories then they form a series, Case 1 with an absolute break between the stem at its bifurcation and the two limbs right and left, Case 2 with its inadequate auriculo-nodal junction and Case 3 without a break in the continuity of the A–V system. In Case 3, though histological examination brought to light no such lesion of the auriculo-ventricular system, like partial or complete break in its continuity, as has been frequently regarded the ultimate anatomical basis of the affection, yet the findings are indicative of such a disturbed circulation of the conducting apparatus and of such an abnormal condition in its neighborhood as might profoundly influence its functional capacity.

Discussion:

Dr. Bishop: The first case reported was one that I had at the Lincoln Hospital. The case was typical for Stokes-Adams Disease. I kept the gross specimen for a number of years, since it was a remarkably beautiful one. The nodules beneath the aortic valve showed plainly, the specimen being preserved without being cut. It is an interesting fact that a young Irish student came into my office one day and told me that my photograph of this heart was being shown in Stokes' clinic in Dublin as a remarkable example of the disease.

Dr. Oppenheimer: That gross examination is inadequate for determining whether or not a lesion lies in the pathway for conduction was illustrated by the findings in a heart given us by Dr. MacCallum. The case was one in which there was no clinical history suggesting heart block; macroscopically the heart showed a calcific nodule in the region of the bundle of His, microscopically the nodule was just alongside of the A–V system and had not compromised it at any point.

WOUND HEALING *IN VITRO*

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As I pointed out in an early communication the lymph and plasma methods of tissue cultivation are modelled after the process of wound healing in the body. Consequently, it seemed logical to assume that an analysis of the factors concerned with the growth of cells in a culture would aid in a better understanding of the process of repair in the body.

A large number of experiments have been performed by several observers including myself for the purpose of determining conditions more favorable for the growth of cells in a culture, hoping through such studies to better the methods for the treatment of wounds. No one has made, however, up to the present time a careful study of the conditions associated with the growth of cells in the control cultures. Such a study became important not only for the light it might throw upon the mechanism of healing in the body but also from the necessity of establishing some definite means of judging the results of further experimentation.

In the present paper I wish to present some observations upon the movement of epithelial cells and, further, to point out some of the changes that occur in the environment of cells during their migration and to show how cellular movement in a culture finds the most satisfactory explanation by the surface tension theory.

As a basis for the first series of experiments I shall consider more carefully some observations made by Ruth in 1910 and 1911 upon the cicatrization of frog skin *in vitro*.

Ruth found that if small holes were cut in pieces of frog skin and these pieces were planted in a drop of plasma the wounds, if not too large, would heal. This healing took place largely from the epithelial layer, the fibrous tissue portions showing no evidence of cellular activity. The epithelial cells moved out from all sides of the wound either as single cells or sheets of cells and eventually covered the wound. Later, the fibrous tissue portions, previously remaining inactive, moved slowly and apparently passively together.

More careful analyses of these wounds have shown that the healing of the epithelial layer was perfect in many cases; no marring remained to mark the site of the original cut. The interesting features to be studied were, first, what force attracted or brought together the inactive fibrous tissue portions, and, secondly, how did the epithelial cells restore themselves so completely to their former shape and arrangement? It seemed evident that the process must have taken place through a form of cellular movement for no cell division was seen in these specimens. That this is true is shown by the following experiment:

A strip of frog skin 5 mm. in length and 1.5 mm. in breadth was cut into five pieces of nearly equal size and placed in a row in a drop of plasma. The distance between each piece was from 0.2 to 1.2 mm. After a few hours liquefaction of the fibrin was noticed by the formation of a small cavity near the tissue border. The cavity slowly increased in size and opened on one or the other surfaces of the clot. Subsequent to the formation of the cavity the germinal layer of the skin moved out as a continuous sheet of cells over the inner surface of the cavity and out onto the surface of the clot. Liquefaction of the clot continued along the sides of the membrane until a deep furrow was formed in the coagulum. The outer border cells of the membrane continued to move outwards clinging to the fibrin at the edge of the furrow while the intermediate cells became free from the fibrin surface. The membrane thus became stretched through an open liquid cavity between the tissue on one side and the solid clot without.

The membrane increased in surface area through a continuous outward movement of its border cells and a stretching more and more of the cells within it. The membrane at first several cells in thickness was soon reduced to a single layer of flattened cells which were elongated in the direction of the movement.

Eventually adjacent portions about each piece met and fused with the membrane of its neighbor. The fibrin about the pieces underwent complete dissolution. The separate pieces then swung in an open liquid cavity by a continuous membrane of cells which were attached to the fibrin wall without.

Growth ceased after a few hours. The cells remained inactive for a time when they finally let loose their fibrin attachment, retracted and took their former position within the tissue fragments. The retraction of the membrane between the tissue fragments brought the fibrous tissue portions together. The strip appeared again the same as before it had been cut into pieces, except for a poor cementing together of the fibrous tissue portions.

This process of healing is the result of an active movement of outer border cells of the epithelial layer and a stretching or a staining of the intermediate cells in the layer. With the release of the force exerted at either end the elastic membrane returns immediately to its former shape.

In order for such a membrane to form, a system of forces working in opposite directions must exist. The tissue must be held firmly on one side and the clot must have sufficient firmness to overcome the stress of the cells moving over its surface. Often one notices that the fibrin is changed into coarse bands as the result of the pull of the cell upon it. In other cases when the fibrin clot finds no attachment to rigid bodies without it is pulled in mass toward the tissue and eventually entirely dissolved.

The same failure of the formation of a membrane is interestingly shown when the tissue fragment is unsupported. A piece of tissue was placed in a drop of fibrin and after clotting had intervened it was cut loose from the clot on all sides except one. This piece of skin showed only a narrow membrane of cells on its attached side. The whole piece of skin on the other hand showed active movement. It traveled several millimeters across the culture, resembling a machine plowing its way through the solid coagulum.

The same phenomenon is seen in wounds where the edges are loosely connected with the underlying or surrounding tissue. Linear wounds heal completely often by a continuous and slow moving together of the walls on the two sides.

The walls of round or rectangular wounds continue to move together by such a process until their edges become more compact and resist the stress of the membrane which now stretches to bridge the gap. Later, an active cellular proliferation increases the number of cells in the membrane allowing them to return to their former shape and thickness.

What has been found true in a tissue culture for the movement of the epithelial cells is likewise true for connective tissue cells. Wounds made in pieces of chicken skin may contract to a fraction of their original size during the first few hours after the preparation of the culture. In many cases activity was seen only in the cellular connective tissue layer. The cells during this contraction were just visible along the border of the tissue. The distal poles of the cells were in contact with the fibrin while the proximal poles remained attached to the cells within the fragment.

That the work necessary to stretch the membrane or move the piece of skin was the result of a decrease in surface tension along the outer edge of the cell membrane became hypothetical through a further study of the factors concerned with such movement. In many of the cultures the outer border cells show evidence of an active amœboid movement. Rounded or pointed processes may be seen to move outward and later to be slowly withdrawn. In other cases the membrane may move a considerable distance maintaining throughout a perfectly uniform straight border. The same is likewise true of connective tissue cells. Frequently

I have seen connective tissue cells move a considerable distance without showing visible changes in their contour or the arrangement of the fat droplets contained within them.

Leo Loeb noted that the epithelial cells in a wound move only when in contact with solid bodies. Harrison analysed this property of cells by the aid of the tissue culture. Harrison found that cells placed in a liquid became spherical and did not move unless they came in contact with some solid surface. In plasma cultures they moved along the fibrin strands, in liquid medium movement was observed along the surface of the cover glass, along strands of spider web or over the free surface of the liquid.

Leo Loeb and Harrison chose to call these movements evidences of stereotropism; *i. e.*, a turning of the cell towards a stimulus given by a solid body—a movement which results from the close apposition of the cell to the stimulating body.

If such were the case, the cell would migrate independent of its neighbors and dependent alone on the contact stimulus. When single epithelial or connective tissue cells are placed in a drop of plasma of uniform consistency, they spread out over its surface but they have never been seen to change their position.

When two cells, on the other hand, are placed close together, they move slowly apart. Cells migrate farther away from a large than a small piece of tissue; farther away from a cellular than a fibrous piece of tissue.

A careful survey of the above observations shows that the changes in the shape of cells as well as their migration find a close analogy to surface tension changes in many other liquid substances—an analogy made by Quinke as early as 1867 in explanation of protoplasmic movement. Quinke assumed that the cell responded much the same as a drop of oil. A drop of oil suspended in water takes a spherical form. A similar drop placed on the surface of water flattens out. The surface tension of the drop immersed in water is equal on all sides. The drop placed on the surface of the water comes, on the other hand, under the influence of three surface tensions: the air-oil surface tension, the oil-water surface tension, and the water-air surface tension.

The first two tend to round off the drop while the last tends to draw it out. Since the water-air tension is greater than the sum of the other two, the drop continues to spread until it covers the entire surface of the water.

The cell through an active metabolism produces continuously changes in the surrounding medium. Food materials are used up and acid waste products are liberated.

It is well known that when fatty acids are brought into solution in water they condense at its surface and cause a lowering of surface tension. When cells are placed in fresh medium they spread at its surface. Subsequent to an active growth and an exhaustion of the medium they become spherical. Remove these cells quickly to fresh medium they spread again at its surface.

These observations give a ready explanation of the migration of cells in a tissue culture. When a fragment of tissue containing many cells is placed in a culture medium an active metabolism of the cells in the midst of the fragment causes rapid changes in the small amount of medium which bathes them. Acid can be demonstrated in the center of the fragment even a few hours after the preparation of the culture. Such a local change results in diffusion currents. The border cells of the tissue must be bathed on one side by a stream of acid waste products and on the other side by an incoming stream of fresh medium. The cell is thus supported by a surface film having a lower surface tension at its proximal than distal part. The cell must then be carried out and away from the tissue mass.

If a piece of connective tissue or spleen be placed in a medium presenting a uniformly free surface on all sides of the tissue fragment, the cells migrate out along such lines of diffusion. They radiate out on all sides of the tissue like light waves from an illuminating body.

There is found in all these cultures a close relation between the rate and extent of cellular migration and the quantity of acid produced. The greatest movement of cells is seen always during the height of the acid production. Later in the history of the culture when less and less acid is liberated in the center of the fragment there is a progressive decrease in the movement of the cells.

Further evidence in support of the surface tension theory is seen in the movement of red blood cells, tissue debris and fat droplets which frequently accumulate in the medium about the tissue fragment. With the onset of activity in the center of the tissue mass these substances move out into the surrounding medium. They follow the same course to be later followed by the moving cells.

I have not presented this theory of surface tension as new. I have given it here in some detail since other theories have been advanced and it seems to give the most plausible explanation of the facts now known about the movement of tissue cells in a culture. It is hoped by the utilization of this theory and these facts that a further and more complete analysis of wound healing can be made.

(Lantern slides)

ON THE SIGNIFICANCE OF THE SUBMILIARY MYOCARDIAL NODULES (ASCHOFF) IN ACUTE RHEUMATIC FEVER

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Aschoff in 1904, at a meeting of the German Pathological Society, described a microscopic lesion in the myocardium of cases of acute rheumatic fever, which he considered specific. Aschoff called these focal lesions the "submiliary nodules of rheumatic fever" and stated that they occur most frequently in the neighborhood of small and middle sized vessels and are composed of large cells with one or more abnormally large, or slightly notched, polymorphous nuclei, the cells being arranged in the form of a fan or rosette. Aschoff believed that the giant cells arose from the adventitial "Wanderzelle." These cells, individually or grouped in nodules, give the rheumatic cell masses their characteristic appearance.

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[1] Work done during the tenure of a George Blumenthal, Jr., Fellowship in Pathology.

Geipel studied seven cases of rheumatic endocarditis and found the Aschoff bodies present in all. He thinks that the characteristic cells of the nodules arise from connective tissue cells of the adventitia or the interstitial connective tissue of the myocardium.

Coombs found the Aschoff bodies in ten of thirteen rheumatic cases. He never found them in non-rheumatic cases. His idea of their origin is the same as that of Geipel who believes that they are converted into fibrous scars, in accordance with the views of Aschoff, Geipel and others.

Wachter found the Aschoff bodies in one case; Bracht and Wachter, in three cases; Sago, in five cases; Takayasu and Low, each in one case.

Fraenkel studied twenty cases in which blood cultures had been made "intra vitam." Eight of his cases gave a rheumatic history and five did not. In seven cases no history was available. Of the eight cases with rheumatic history five showed Aschoff bodies. In the seven cases in which no history was obtainable, but which showed verrucous endocarditis, Aschoff bodies were present in six. In the five cases in which there was no history of rheumatism, nodules were present in two cases, absent in two cases and in the fifth case, which was one of chorea, characteristic nodules were also present. Fraenkel believes that even in the absence of a history of rheumatism the finding of Aschoff bodies is strong presumptive evidence of a preceding rheumatic infection. In the hearts of patients dying from various types of bacterial endocarditis, Aschoff bodies were not present. Fraenkel demonstrated the value of the methyl-green pyronin stain for differentiating Aschoff bodies from other foci.

Douglas found Aschoff bodies in one case of acute rheumatic fever, and Huzella in six cases of acute rheumatic fever and in one case of chorea.

Material Studied

We have studied the hearts of three cases of acute rheumatic fever; of three cases of chorea without rheumatic history; of one case of combined verrucous and subacute bacterial endocarditis without rheumatic history; of fourteen cases of subacute bacterial endocarditis in the bacterial stage of the disease; of five cases of subacute bacterial endocarditis in the bacteria-free stage; of five cases of chronic valvular disease with no rheumatic history; of five cases of acute streptococcus endocarditis; one case of pneumococcus; three of gonococcus and three of staphylococcus endocarditis.

CASES SHOWING ASCHOFF BODIES

We have found Aschoff bodies in the three cases of acute rheumatic fever and in the case of combined verrucous and subacute bacterial endocarditis. One of the cases of chorea showed great numbers of Aschoff bodies, while in the other two cases, in which the disease existed for only a short time before death, only a few Aschoff bodies were present.

CASES WHICH DID NOT SHOW ASCHOFF BODIES

Aschoff bodies were found in only one of the remaining cases. This was a case of subacute bacterial endocarditis in the bacterial stage and the patient had suffered from an attack of acute articular rheumatism eleven years, and one year, before death. The heart showed fibrous scars about the small vessels and in some of these scars a few of the characteristic cells of the Aschoff bodies.

CONCLUSIONS

(1) In rheumatic myocarditis foci termed submiliary nodules of Aschoff are present which are characteristic of the rheumatic infection.

(2) They are most frequently found in the walls of the left ventricle, the auricles usually escaping.

(3) The nodules were found in three cases of chorea without joint manifestations, proving the close relationship of this condition to rheumatism.

(4) They were absent in fourteen cases of subacute bacterial endocarditis, variety streptococcus mitis (viridans).

(5) They were not found in infections of the endocardium with the gonococcus, staphylococcus, streptococcus or pneumococcus.

(6) Even in the absence of arheumatic history he believe, in accordance with Fraenkel, that the presence of Aschoff bodies signifies a previous rheumatic infection.

(7) Aschoff bodies are not always found in rheumatic carditis, where the infection antedates death by a long period, but the healed remains, represented by sclerotic patches (Schwielen), are present.

(8) We would suggest that the cases of arthritis characterized by the presence of the "submiliary nodules of Aschoff" in the myocardium be placed in one group and called for the time being "rheumatism." Those cases with articular manifestations, yielding positive bacteriological findings and with absence of Aschoff bodies, should not be classified as "rheumatism," but according to the infecting organism.

THE EXPERIMENTAL PRODUCTION OF FOCALIZED MYOCARDIAL LESIONS WITH STREPTOCOCCUS MITIS (VIRIDANS)[1]

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There are numerous ideas current as to the etiological agent in acute rheumatic fever. Many observers have failed to cultivate bacteria from the blood and joint exudates of these cases and do not believe in the bacterial origin of this disease. The ideas of those who do believe in the bacterial origin can be divided into four groups: (*a*) That rheumatism is caused by common pyogenic bacteria; (*b*) That it is caused by a diplo-streptococcus as described by Leyden and to which Triboulet gave the name of

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[1] Work done during the tenure of a George Blumenthal, Jr., Fellowship in Pathology.

"*Streptococcus rheumaticus*"; (*c*) That the bacterial agent is a Gram-negative, anaerobic bacillus known as Achalme's bacillus; (*d*) That the disease is a mixed infection of cocci and bacilli, perhaps not even specific.

Our interest in this subject has been aroused by two things, first, the similarity of the cultural characteristics of *Streptococcus rheumaticus* of Leyden, Triboulet, F. Meyer, Wassermann, Poynton and Paine and others, and *Streptococcus mitis* (*viridans*) of Shottmüller, with the *Streptococcus mitis* which has been isolated with great frequency at this laboratory from the blood of cases of subacute bacterial endocarditis which have been studied by Dr. Libman. The cultural characteristics of these organisms will not be gone into here since this phase of this work has been taken up by Drs. Libman and Aschner. Second, because of the recent work of Coombs and Leila Jackson, who claim to have produced experimentally with *Streptococcus pyogenes*, *Streptococcus rheumaticus* and *Streptococcus mitis* (*viridans*) focalized myocardial lesions either identical with or resembling Aschoff bodies.

Our work was undertaken with the idea of discovering the nature of the myocardial lesions produced with *Streptococcus mitis* from cases of subacute bacterial endocarditis, and not with the idea of the investigation of this organism as the possible etiological agent of rheumatism.

A number of observers have produced a vegetative endocarditis in rabbits by intravenous injection of *Streptococcus mitis*. In addition Bracht and Wachter have described definite histological lesions in the myocardium.

Carey Coombs working with *Streptococcus rheumaticus* describes the production of Aschoff bodies in rabbits. Leila Jackson produced focalized myocardial lesions in rabbits by the intravenous injection of streptococci isolated from patients in the Chicago sore throat epidemic.

ANIMAL EXPERIMENTS

Nine different strains of *Streptococcus mitis*, five strains of *Streptococcus rheumaticus* and three strains of the streptococcus of epidemic sore throat were used.[1] The organisms were grown on serum agar, serum glucose agar, blood agar and bouillon. Saline suspensions were made from twenty-four hour agar cultures and the growth in bouillon was concentrated by centrifugalization. The dose varied from twenty-four hour growth on one slant agar tube to that on two Blake bottles and the sediment of growth in from 10 to 100 c.c. of bouillon.

Rabbits were used for these experiments and received from one to sixteen injections at intervals of from three days to several weeks. Forty-two rabbits were injected with *Streptococcus mitis.* All of these showed the microscopic lesions described below. Seven per cent. of these animals developed vegetative endocarditis. Twenty one per cent. developed hemorrhages in the cardiac valves.

MYOCARDIAL LESIONS

Most of the hearts showed no macroscopic lesion, except those mentioned above, but a few showed grayish streaks in the myocardium.

Microscopically, a progressive type of lesion was found, depending on the number of injections and the length of time the animal lived. The earliest stage is represented by small areas including only a few muscle fibers showing slight degenerative changes and between and about them a few infiltrating lymphocytes, large mononuclear leucocytes and occasionally plasma cells and only rarely a very few polymorphonuclear leucocytes. In animals which have received many injections these areas are considerably larger, and in these the degenerating fibers at times show marked fragmentation, the surrounding fibers being normal. The degenerating muscle fibers take a slightly more intense eosin stain than normal and the fibrils disappear rather early, leaving a homogeneous, finely granular protoplasm with small and large vacuoles. Some of these fibers are slightly swollen, but most are atrophic. Their nuclei are irregular and either pale and vesicular, or small and pyknotic, and show no tendency to proliferation. In addition to these more common lesions some of the hearts which show grossly grayish streaks in their myocardium show microscopically large and small areas of complete necrosis of the muscle fibers with calcareous infiltration and a slight round cell inflammatory reaction about them. With the atrophy of the muscle fibers, the interstitial tissue stands out more prominently and very early begins to proliferate, fibroblasts appearing. These areas are irregular in shape but usually elongated, with their long diameter parallel to the muscle fibers.

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[1] We are indebted to Dr. Winslow, of the Natural History Museum, New York, for three strains of *Streptococcus rheumaticus;* to Dr. Rosenow for two strains of *Streptococcus rheumaticus* and three strains from the Chicago sore throat epidemic.

From this stage on the lesions partake more of a proliferative connective tissue character than of an infiltrative leucocytic or degenerative myocardial one. In none of these areas are many degenerating muscle fibers seen, but fibroblasts soon become prominent and develop rapidly to a fibrous stage. Coincidently with this the round cell infiltration decreases until finally there results an area of fibrous tissue surrounded by healthy muscle fibers and containing only a few leucocytes. All of these fibrous areas are not circumscribed; many are diffuse, their fibers running parallel to those of the myocardium, apparently having replaced the latter. Some of the focalized lesions bear a close relationship to the small and middle sized blood vessels, but many lie scattered through the myocardium, occurring in the greatest numbers near the base of the ventricles and in the papillary muscles. In some animals the small blood vessels show an intimal proliferation or a connective tissue proliferation immediately about them. Occasionally hyaline thrombi are found in them, but these do not occur with any regularity and seem to have no relationship to the lesions described above. These lesions show no predilection for a subendocardial situation nor the bases of the cardiac valves, and no proliferation of the endothelium of the endocardium or valves is found. No multinuclear cells such as are described by Leila Jackson, or peculiar cells with large vesicular nuclei and scanty protoplasm as described by de Vecchi, are present. With the methyl-green pyronin stain on alcohol fixed material the fibroblasts, and in some hearts the endothelium of the blood vessels, stained a bright red as did some of the degenerating muscle fibers. No structures at all resembling the submiliary myocardial bodies of Aschoff are found and the red staining of the fibroblasts etc., with the methyl-green pyronin has an entirely different tint from that taken by the cells of Aschoff bodies. These latter stain a deep rich red and the fibroblasts a bright red, not so deep and with a highly refractile quality, giving them an appearance which for lack of a better term we call translucency. In none of the hearts, although carefully searched for, were bacteria found either in association with the lesions or elsewhere, except in those hearts where vegetative endocarditis was present. Here streptococci were found diffusely scattered in small clumps in the capillaries between normal muscle fibers and also in some of the lesions, but not in all the lesions in these three hearts.

Eleven animals injected with *Streptococcus rheumaticus* showed the same microscopical changes as above. Seven animals injected with the streptococcus from epidemic sore throat showed degenerative and destructive lesions with leucocytic infiltration and the presence of great numbers of cocci. A few fusiform areas, some resembling those described by Leila Jackson, were found, but without giant cells and in no way resembling Aschoff bodies.

DISCUSSION

The microscopic myocardial lesions produced with *Streptococcus mitis* are identical with those produced with *Streptococcus rheumaticus* and those produced by Bracht and Wachter with organisms having similar cultural characteristics. These lesions are entirely different from the nodules described by Aschoff.[1] In view of the fact that *Streptococcus mitis* has been recovered from the cardiac vegetations and from inflamed rabbits' joints a considerable time after they first appeared and because streptococci were not found in the focalized myocardial lesions, we lean toward the idea that these focalized myocardial lesions were caused by bacterial toxins rather than by the organisms themselves. The capacity of *Streptococcus rheumaticus* to produce myocardial focalized lesions can be looked upon simply as a property which this organism shares with other types of streptococci and is in no way specific.

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[1] Professor Aschoff examined our specimens and agreed with our opinion on this point.

CONCLUSIONS

(1) By the intravenous injection of *Streptococcus mitis* in rabbits we have produced focalized myocardial lesions which are identical with those caused by injection of *Streptococcus rheumaticus*, and those produced by Bracht and Wachter with a *Streptococcus viridans* type of organism.

(2) These lesions are different from those which we produced by injections of streptococci from the Chicago sore throat epidemic (epidemic streptococcus).

(3) The lesions are not Aschoff bodies and are easily differentiated from them. They also differ from lesions produced by Leila Jackson and Carey Coombs, which these authors describe as either Aschoff bodies or similar to these bodies.

(4) These myocardial lesions in rabbits appear to be caused by toxins liberated by the organisms injected and not by the organisms themselves.

(5) The only similarity between these experimental lesions and those found in cases of rheumatic carditis is the focalized nature of both.

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DR. A. B. WADSWORTH, *President*

A NEW HEMOGLOBIN AGAR MEDIUM FOR THE CULTIVATION OF B. INFLUENZÆ

WM. THALHIMER, M.D.

(From the Pathological Laboratory of the Mount Sinai Hospital, New York City)

Pfeiffer showed, originally, that it was necessary to have blood present in order to successfully cultivate *B. influenzæ*. He then proved that the necessary constituent of blood was hemoglobin. Since this time various investigators have attempted to cultivate this organism on media containing various substances beside blood, such as hematin, hematogen, yolk of egg, egg albumin, serum albumin, globulin from blood fibrin, cholesterin, mucin from bile, various albumoses, semen of animals, etc. On some of these media slight or moderate growth of this organism has been secured but these results have not been constant in different hands, and none of them can be considered adapted for laboratory cultivation of *B. influenzæ*.

In a previous article the author reported an unsuccessful attempt to grow *B. influenzæ* on a medium prepared from a crystalline hemoglobin product obtained from Armour & Co., through the courtesy of their laboratory. In this article was reported the successful cultivation of this organism on a medium prepared from freshly drawn beef blood obtained from an abattoir.

The method for preparing this medium is as follows: Defibrinated beef blood is laked by the addition of an equal part of distilled water and rendered free from bacteria by passing through a sterile Reichel filter. This yields a clear, red fluid and from 20 to 40 c.c. of this is added to a litre of sterile, melted agar at 45° C., poured in sterile tubes and slanted. The medium which results is perfectly clear, bright red and of the same density of color as ordinary blood agar usually used in the laboratory.

The unsuccessful medium made with the crystalline hemoglobin was prepared in a somewhat similar manner. A solution of the hemoglobin was made in distilled water, yielding a dark brown fluid, filtered through a Reichel filter, the resulting filtrate added to melted agar giving a brown medium of about the same density of color as the medium above described.

Since the time of the above investigations two amorphous preparations of hemoglobin were obtained and media prepared according to the above method, and various strains of *B. influenzæ* have been successfully cultivated on this medium, some of them for as many as twenty-one generations. The growth was more luxuriant than on blood agar.

By means of either one of the two successful media above described any laboratory can cultivate *B. influenzæ* without the necessity of starting with sterile blood in the manufacture of media. The medium prepared from amorphous hemoglobin is of interest only because with a hemoglobin product chemically prepared, and far removed from the original blood, a luxuriant growth of *B. influenzæ* can be obtained, demonstrating that hemoglobin and oxyhemoglobin in their original form are not necessary for the successful cultivation of this organism.

Discussion:

Dr. Wadsworth: How long will the hemoglobin solutions keep after filtration? Will they keep for weeks or months after passing through the Berkefeld filter?

Dr. Williams: What proportion of hemoglobin is used? The research laboratory of the health department has been working with hemoglobinophilic organisms for some time and we have found that some organisms will grow luxuriantly on a proportion of one part of horse blood to five hundred of veal broth if the horse blood is added to hot agar at 90° C. and allowed to cool immediately at room temperature. We have used this medium for stock solutions and for making antigens.

Dr. Thalhimer: In reply to Dr. Wadsworth, the solution of hemoglobin will keep well enough to be used several weeks after filtration. I have grown organisms successfully at this time and have never tested the solution at longer periods. I have not yet worked out the minimum amount of the filtered solution of commercial hemoglobin that can be successfully used, but know that one can get growth in a medium which is scarcely colored by the hemoglobin which is added. In the test tubes which are being passed around are found three kinds of media. Two are made up with a hemoglobin solution prepared from sheep's blood; one of these is dark brown in color and the other one scarcely tinted at all; the third kind of medium is made up with commercial hemoglobin and also contains an amount which scarcely colors it. On all three media the growth can be seen to be perfectly satisfactory.

THE VIABILITY OF THE BACILLUS PESTIS IN STOCK CULTURES

ROBERT J. WILSON, M.D.

At the meeting of the New York Pathological Society of December 13, 1899, Dr. W. H. Park presented cultures of *Bacillus pestis* isolated from two of the crew of the steamship *J. W. Taylor* which arrived at this port from Santos Brazil on November 18 of that year. At this time studies of these organisms were being made at the research laboratory of the department of health, and the keeping up of the stock was in my charge. Our rule was to transfer the cultures at monthly intervals.

On June 8, 1903, I had a number of cultures that had been kept in the ice box unopened from February 21, 1901, and I was surprised to find that on subculture from these the organisms grew almost as readily as from recently planted cultures. At this time I made a number of cultures of three strains of *Bacillus pestis* with the idea of transferring them at yearly intervals for a period of several years.

On November 14 of this year I made a transfer from a culture tube that had not been opened since June 8, 1903, a period of ten years and five months. After forty-eight hours there was an abundant growth of organisms which upon subculture proved to be typical pestis bacilli. These organisms proved virulent to a guinea-pig after forty-eight hours, and the pestis bacillus was recovered in pure culture from the heart's blood of the animal. This longevity of the organism is not confined to one strain, for I have a transplant from a culture nine and a half years old which was originally obtained from Kitasato's Laboratory, the culture having been isolated by him from a case in Hong Kong, China.

Discussion:

DR. WADSWORTH: Nine and a half years is certainly an unusual length of time to keep the plague bacillus alive. In my experience it often dies very rapidly. The late Dr. Hiss used the method of sealing culture tubes. He kept typhoid bacilli alive for many years and many other species may be kept alive that way. In 1899 in the Institute for Hygiene in Berlin stock cultures were kept sealed. The organisms remain alive in a constant biological state. It is certainly very interesting to know how long an organism such as the plague bacillus can be kept alive by this method.

THE IMPORTANCE OF THE AROMATIC AND HETEROCYCLIC COMPLEXES IN SPLIT PRODUCTS OF PROTEINS FOR THE PRODUCTION OF THE ANAPHYLAXIS-LIKE PHENOMENA OF PEPTON "SHOCK"

GEORGE BAEHR, M.D.

(Assistant in Pathology, Mount Sinai Hospital, New York)

As is undoubtedly known to everyone here, the intravenous injection into animals of the products resulting from the peptic digestion of non-toxic proteins is followed by symptoms which closely resemble those of anaphylactic shock. This observation of de Waele, and of Biedl and Kraus has lent considerable support to the theory that anaphylactic shock is directly or indirectly due to the liberation of such peptons by the parenteral digestion of the injected protein. It is not my purpose to enter into any discussion of the relative merits of the different theories of anaphylaxis. I merely wish to present some work done in conjunction with Ernst P. Pick of Vienna, which would seem to narrow down the responsible substance in the production of the so-called pepton shock to a still smaller portion of the protein molecule.

The work was suggested by the observation of Chittenden, Mendel and Henderson that the products of the peptic digestion of gelatin are practically inactive. As is well known, gelatin is a protein which is deficient in aromatic and heterocyclic complexes,—in complexes in which the carbon and other atoms are arranged in ring formation. This suggested the possible importance of these groups, particularly as Obermayer and Pick were able to show that chemical changes affecting the cyclic nucleus of a protein modify its antigen properties.

On the other hand, Schittenhelm and Weichardt have recently claimed that just those proteins which are rich in diamino-acids upon peptic digestion gave rise to the most active peptons, whereas the peptons from proteins containing chiefly or entirely monoamino-acids are biologically absolutely inactive. In fact de Waele is sponsor for the viewpoint that the pepton action is essentially an amino-acid action. Such an hypothesis is of course immediately contradicted by the fact that the pepsin ferment which liberates the toxic products does not possess the power of splitting off amino-acids, whereas trypsin which does split off amino-acids destroys the specific toxic property of the peptic digestive products.

In order therefore to determine which portion of the protein was the essential factor in the production of the pepton shock, we subjected it, first, to procedures which would produce chemical changes in its cyclic complexes and, secondly, to procedures affecting only the amino-acid groups.

Either horse or beef serum was always used and with this iodine protein preparations were made according to Hofmeister's method, nitrate protein preparations by the method of v. Fürth and diazo protein by the method of Obermayer. I shall not go any further into the technical details or the chemistry involved, but shall refer those interested to a paper by Pick and myself in a recent number of Schmiederberg's Archiv. Suffice it merely to say that by each of these procedures a substitution with either iodine, nitrate, or diazo radicals is effected chiefly in the aromatic, partly also in the heterocyclic nucleus of the protein, in other words in the benzol, imidozol, indol complexes, etc.

After proper purification, the preparations were subjected to thorough peptic digestion. Chemical analyses of the resultant split products showed that the serum proteins had been completely broken up into their primary and secondary albumoses and into their peptons. The latter gave an intense Biuret reaction and was precipitable by tannic acid and by all the other alkaloid reagents. But the Millon reaction both with the iodid, nitrate and diazo digestive products was absolutely negative, thus indicating a complete saturation of the aromatic complexes in the protein.

These pepton preparations were then tested upon animals. Ordinary peptons obtained from the peptic digestion of animal proteins, such as Witte pepton, when injected intravenously into dogs produce amongst other things an incoagulability of the blood and a sudden and severe drop in blood pressure which persists for a long time, or until death supervenes. Should the animal recover, immunity to subsequent doses of the poison persists for some hours. In guinea-pigs also the injection is followed by a picture resembling that of the anaphylactic phenomenon, the chief characteristic of which is a sudden spasm of the bronchial musculature. The animal has several jumping convulsive seizures and dies, probably of asphyxia. Perfused through the hind legs of a frog, the pepton manifests a marked dilator action.

The results of the biological tests obtained with the iodid, nitrate and diazo peptons all agreed. In doses several times the amount ordinarily used in eliciting the pepton shock with Witte pepton, all three preparations were practically inert. No drop in blood pressure was produced in dogs and following the administration the blood not only remained perfectly coagulable, but its coagulability was usually very markedly increased. In guinea pigs the results were also remarkable. As much as two grams of any of the three preparations injected intravenously were absolutely incapable of producing the slightest signs, either clinically or microscopically, of any bronchial spasm.

Only one of the pharmacological actions of pepton persisted in the preparations and that was the dilator action on frogs' blood vessels. Perfused through the hind legs of a frog according to the method of Läwen and Trendelenburg, the blood vessels which had been previously contracted with adrenalin promptly dilated. This conclusively demonstrates that the vasodilator action on frog's vessels depends upon an entirely different chemical basis than does the phenomenon of the usual pepton shock.

Perfused through the isolated frog's heart according to William's method, the nitrate and diazo preparations even in one per cent. solutions were absolutely inert. The iodine peptons on the other hand presented merely a toxic iodid action, the heart stopping in diastole.

So much then for the biological action of peptons in which changes in the cyclic complexes had been effected. It could not be concluded as yet that these changes alone were responsible for the biological inactivity of the pepton. For it was still to be determined whether the amino-acid radicals might not also play a part, as had been claimed by de Waele and by Schittenhelm and Weichardt.

In preparing the nitrate proteins it was necessary amongst other things to use nitric acid. According to von Slyke, by the action of nitric acid the amino-acid groups are so thoroughly split off that he has utilized this for his method for the quantitative determination of these groups. It might be argued that in the course of the procedures through which we had put our peptons, the toxic action had been lost because amino-acids had been split off.

It was therefore necessary to exclude these groups alone, without at the same time affecting the cyclic nucleus of the protein. And this was accomplished with formaldehyde which, as is well known, combines with the amino-acids to form stabile methylen compounds. The injection into animals of the peptic digestive products of such formalized protein resulted in all the phenomena characteristic of pepton shock. In its effect upon the blood pressure, the blood coagulability, the bronchial musculature, etc., it proved itself to be just as powerful as Witte pepton.

In other words, the evidence seems to be complete that it is not the amino-acid groups, but the cyclic complexes in the protein which are responsible for the production of the anaphylaxis-like symptoms of pepton shock. The intact protein itself is nontoxic because in it all valencies of these cyclic groups are mutually saturated. Upon peptic digestion they become more or less isolated, are apparently unsaturated and hence develop their biologic activity. Combination with iodine, nitrate, or diazo radicals saturates these free valencies and thus also renders the peptons inactive, just as is the intact protein.

Discussion:

Dr. Ottenberg: It occurs to me as interesting in connection with Dr. Baehr's observations that Obermeier and Pick themselves were able by diazotizing, iodizing or nitrating proteids to produce specific precipitins which would act only on the particular kind of chemically altered proteid used to induce the production of the precipitin. In fact, by treating an animal's own blood serum in any one of these ways they were able to produce precipitins specific for the special kind of chemically altered proteid of that kind of animal. In this instance the union of something else with the aromatic ring then altered the specific character of the proteid.

Dr. Pollitzer: A few words about the earliest observations on the effects of the first split products of the albumens when injected directly into the circulating blood may be of interest from a historical point of view. In 1883–84 Schmidt-Mülheim and Fano working in Ludwig's laboratory found that injections of a solution of Witte's pepton produced the effects with which we are now all acquainted. Kühne and Chittenden early in 1884 isolated from Witte's pepton a series of hemi-albumoses. In the autumn of that year I was working in Kühne's laboratory and it was suggested that I repeat the injection experiments, using the various albumoses prepared by the methods of Kühne and Chittenden. I found (*Journal of Physiology*, 1886) that all the various albumoses produced similar symptoms in dogs, but in somewhat different degrees, hetero-albumose injections being often fatal, while the substance we then called pepton, though it produced a great fall in blood pressure did not kill the animal, possibly because of its great diffusibility and rapid elimination through the kidneys. Brieger at this time was engaged on his first most important work on the ptomains, and was of the opinion that the phenomena observed were due to the presence of ptomains which he thought might readily develop in the mixture of proteid substances which constituted Witte's pepton. I showed this view to be baseless by making an amyl-alcohol extract of Witte's pepton to remove possible ptomains and getting the same effects with the residue as with the whole pepton. This question ten years later was the subject of a controversy between Brieger and Salkowsky, who were evidently unacquainted with my work.

CHOLESTERINEMIA

EDWIN HENES JR., M.D.

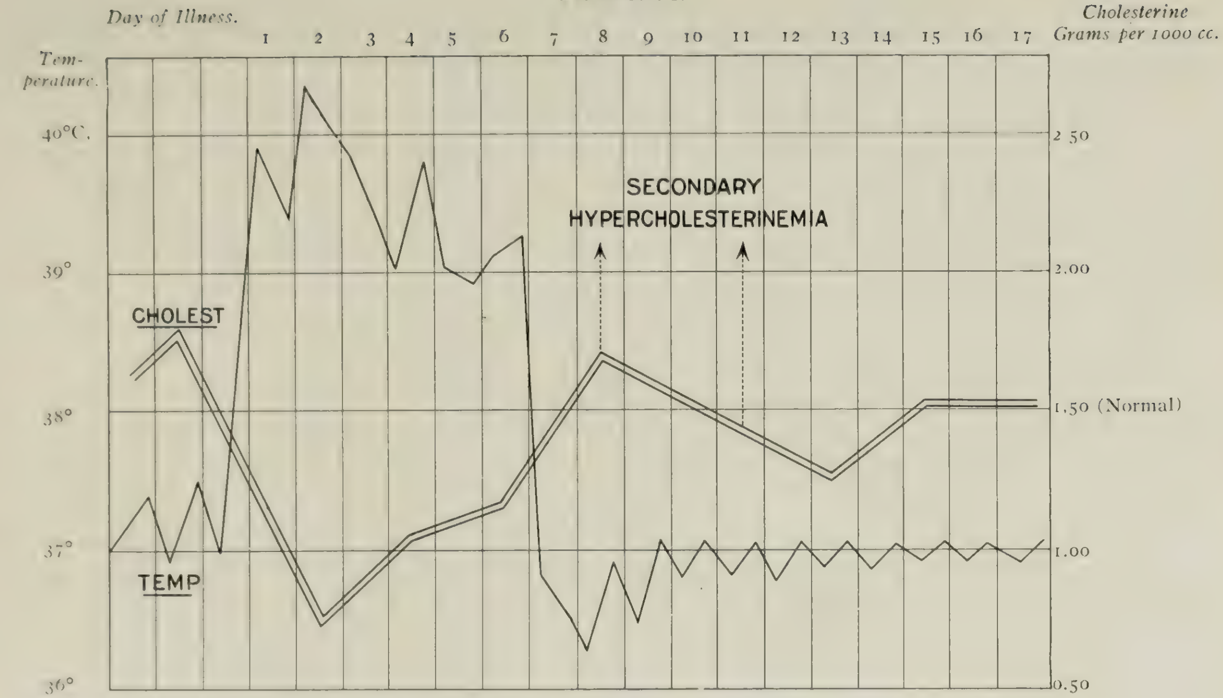
Under the title "The Cholesterine Content of Human Blood Under Various Pathological Conditions," I published in the *Deutsche Archiv für Klinische Medicin* (Band III, Heft. 1 2) the results of a six months' investigation of this subject, carried on at the University of Freiburg, Germany, under the direction of Prof. Dr. O. de la Camp, and with the assistance of Professors Windaus and Autenriedth of the Chemical Institute at Freiburg. Desirous of submitting my results to the English speaking profession and of publishing facts which do not appear in the original manuscript, I have prepared the following less detailed paper under the above title.

Cholesterine has, almost suddenly, aroused intense interest. Up to last winter, little was to be found in the literature relative to the role or function it plays in various pathological conditions. The French, especially Chauffard and his school, had contributed most of our knowledge on the subject. Since then, veritable outbursts of interest and enthusiasm have arisen both here, in New York especially, and in Germany.

I shall not go into the history of cholesterine at this time, other than to say that it was, apparently, discovered in 1775 by Conradi. Austin Flint's contention in 1862 that cholesterine is the product of the metabolism of nervous tissue, has only recently been disproved (1). And Hoppe-Seyler's (2) statement that "cholesterol is purely an excretion and is not reabsorbed, but passes out of the body with the feces," is, according to the excellent work of Dorée, Fraser, Ellis and Gardner ((3), erroneous According to Hoppe-Seyler, "cholesterol is a cleavage product constantly formed in the metabolic changes of the living cell, and for this reason it is that cholesterol is invariably found as a constituent of both animal and vegetable cells." The work of Dorée, Gardner, Fraser and Ellis seems to show quite conclusively that cholesterine is a constant constituent of animal cells, is not synthesized in the organism, is not an excretion, is reabsorbed (probably with the bile salts) and again utilized in new cell formation; and its amount in the blood (other things being equal) depends upon the amount taken in the food, any excess, beyond the possible utilization of the organism, being excreted in the feces. This excretory product termed "stercorine" by Flint, has since been named "coprosterol."

In carrying on my work, I made use of the extraction method as advocated by Weston and Kent (4) and for the quanti-

CURVE NO. 1.



TEMPERATURE AND CHOLESTERINE CURVES IN A CASE OF LOBAR PNEUMONIA.
(From Chauffard, Lareche and Grigaut.)

CURVE I. Note the rapid fall in cholesterine with rapid rise of temperature, and the rapid fall in temperature with the comparatively rapid rise in cholesterine. Hyper-cholesterinemia continued for four days. Temperature had endured only seven days. This is a typical curve.

tative determination of cholesterine, I employed the colorimetric method devised by Grigaut (5), which is dependent upon the Lieberman-Burchard color reaction. This "working combination" I found most serviceable, and, through the kindness of Professors Windaus and Autenriedth, exceedingly accurate and reliable. Our resulting figures, each using a different method,—Windaus, his digitonin method, and Autenriedth, his own colorimetric method,—were almost identical. As a result of this checking up, Autenriedth has added a standard "cholesterine prism" to his set of colorimeters.

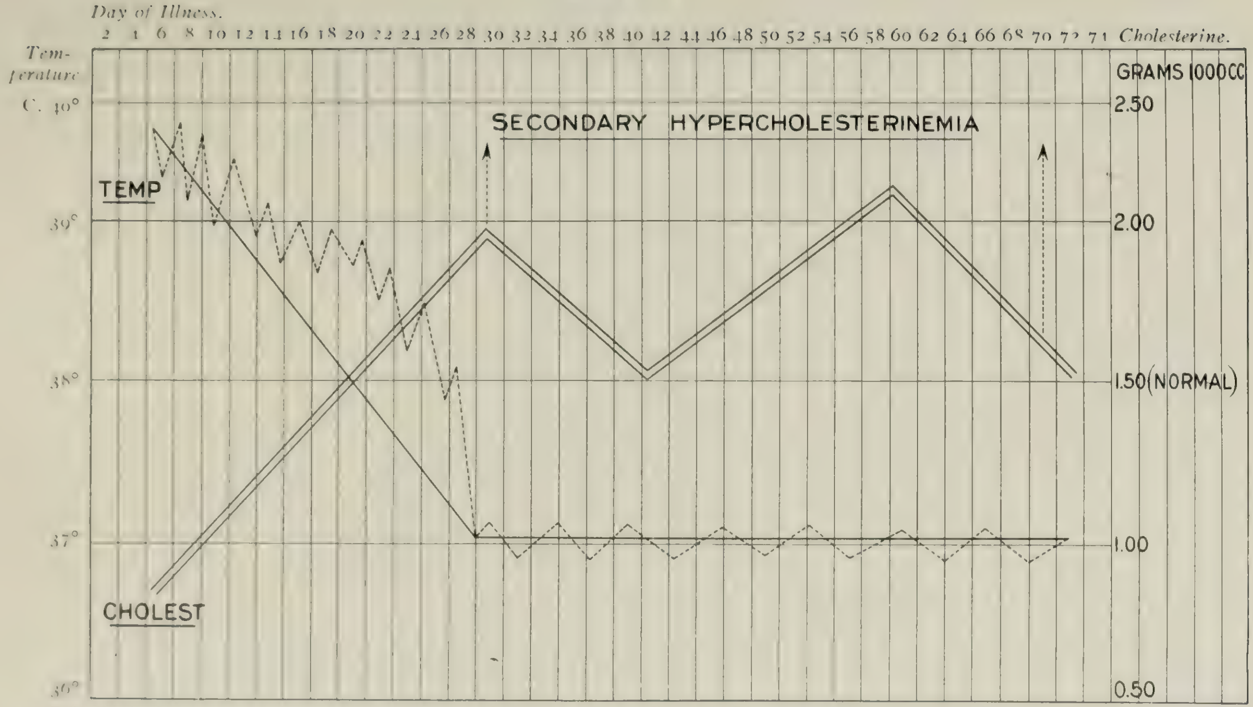
Other methods for the quantitative determination of cholesterine have been recommended, but because I found the Weston-Kent-Grigaut combination so satisfactory and apparently accurate, I used it in making all my determinations.

In selecting cases for the study of cholesterinemia, it is apparent that the difficulty of being unable to find pure uncomplicated cases, is constantly present. The various clinical findings present at the time the blood is taken for examination, must be taken into consideration, for I found several factors which influence the cholesterinemia, notably temperature. The clinical features which I found to influence the cholesterine content of the blood are the following: *Decreasing:* Temperature, old age, emaciation and poor general condition, and diet. *Increasing:* Complicating nephritis, adipositas, arterio-sclerosis, diet and jaundice.

It might be asked in advance, "Shall we use whole blood or serum alone, in making quantitative cholesterine determinations?" My answer is, "We can use either, but in making a series of examinations on the same case, we must, of course, use either the whole blood, or the serum alone, in each examination, for the amounts found in whole blood and in serum are different."

My series of cases number over a hundred, and in each case without exception, I found more cholesterine in a definite amount of serum alone than in the same amount of whole blood. This held true both under normal and abnormal conditions and under conditions of hypo- as well as hyper-cholesterinemia, whether

CURVE NO. 2.



TEMPERATURE AND CHOLESTERINE CURVES IN A CASE OF TYPHOID FEVER. (UNCOMPLICATED.)

CURVE II. Note the gradual fall in temperature and the gradual rise in cholesterine. A hyper-cholesterinemia continued for a long time. The diet during convalescence was not sufficiently rich in cholesterine to account for the steady increase in the cholesterinemia.

physiological or pathological. The percentage increase of the amount found in serum as compared with that found in whole blood varied considerably. Whether this variable percentage increase is of any special significance under pathological conditions, I am not prepared to say. In the various anæmias and other blood diseases, in which the corpuscular elements of the blood undergo retrograde morphological changes, it is possible that the percentage increase above referred to is of significant interest; for the blood corpuscles alone contain considerable of the total blood content of cholesterine, and a destruction of the corpuscles must increase the cholesterine content of the serum.

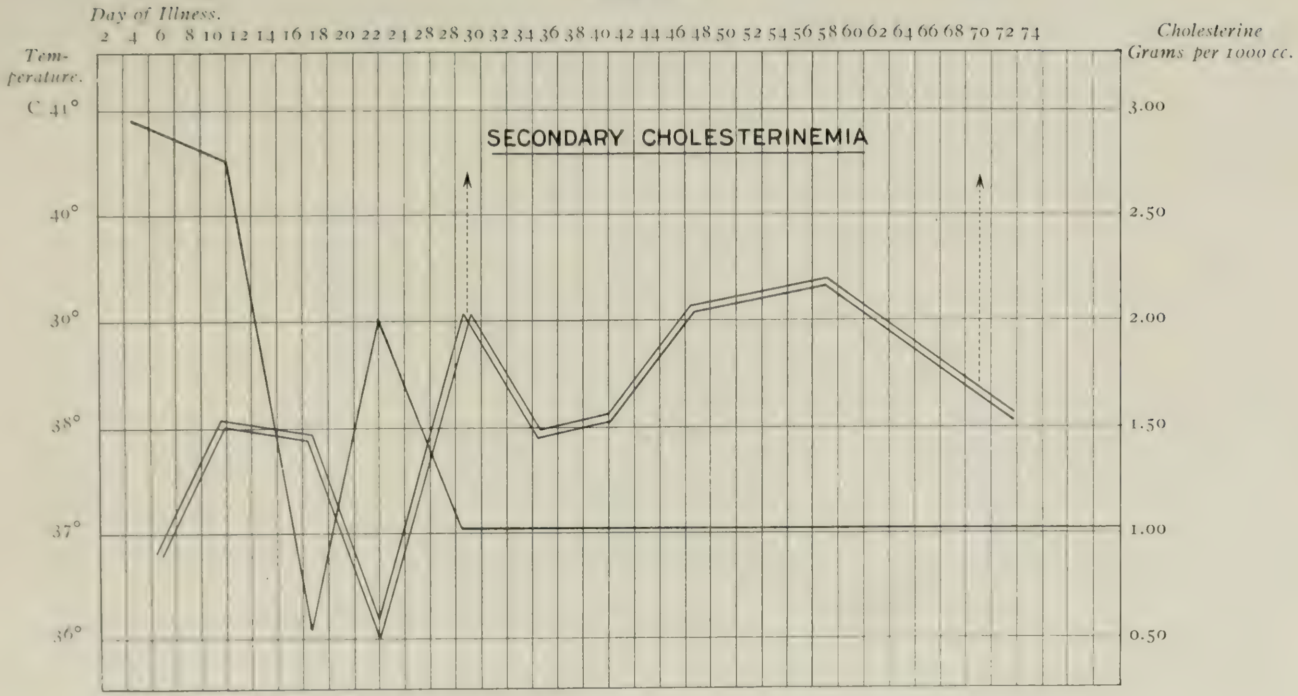
The normal cholesterine content of human blood varies according to a number of authors, between 1.10 and 1.95 grams per 1,000 c.c. Of nine cases examined, males and females, ranging from fourteen to thirty-seven years of age, in whom no influencing factors were present, I obtained figures ranging from 1.10 to 1.82 grams per 1,000 c.c.—an average of 1.48.

A diet rich in cholesterine increases the cholesterine content of the blood. In four normal cases, after a fasting period of four hours the cholesterine content of the blood rose from 1.42 to 1.90, from 2.0 to 2.23, from 1.60 to 1.82, and from 1.40 to 1.56 grams per 1,000 c.c. respectively, after a meal consisting of 100 300 grams of calf's brain, 3 to 4 eggs and 0.5 gram of pure cholesterine. The second estimation was of the blood withdrawn six hours after the ingestion of the above meal. The inconstant increase after the meal is readily explained by the fact that the patients consumed variable amounts of the meal offered. In this connection it would have been of importance to have determined any possible increase in the excretion of coprosterol in the feces. I entertain the belief that under normal conditions of health, the body assimilates only a definite amount of the cholesterine taken with the food; there is, in my opinion, a utilization limit.

In order to determine the comparative cholesterine content of whole blood and blood corpuscles, respectively, I proceeded as follows:

1. 4 c.c. of whole blood were analyzed as usual.

CURVE NO. 3.



COMPOSITE TEMPERATURE AND CHOLESTERINE CURVES IN THREE CASES OF TYPHOID FEVER

CURVE III. Note the relationship between temperature and cholesterinemia. After the thirtieth day all three cases were convalescing. One case had a severe nephritis of short duration which accounts for the relative hyper-cholesterinemia.

2. 4 c.c. of the same blood were repeatedly centrifuged and the supernatant serum pipetted off and analyzed.

3. 4 c.c. of the same blood were washed four times in normal salt solution and the corpuscular sediment alone analyzed. In each of five cases 1 c.c. of serum always contained more cholesterine than 1 c.c. of whole blood. The serum of 1 c.c. of blood always contained more than the corpuscles of 1 c.c. of the same blood. On an average the corpuscles alone, according to my figures, contain 44.4 per cent. of the total cholesterine content of whole blood.

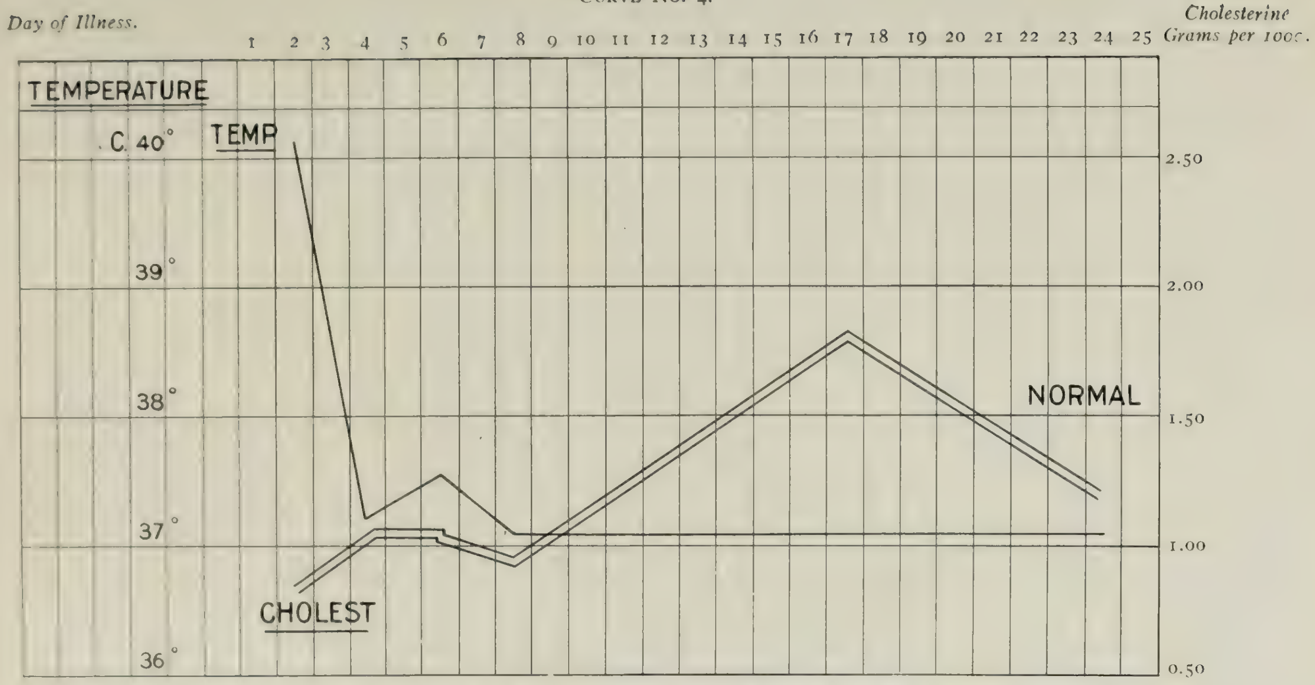
I sought further to determine to what an extent one or the other or all of the separate blood constituents are influenced in their cholesterine content by the production of a physiological hyper-cholesterinemia. Again, as previously, a diet of calf's brain, eggs and 0.5 gram of cholesterine was given. Before the meal the average cholesterine content of the corpuscles alone as compared with that of the whole blood was 47 per cent. After the meal this percentage was 46⅓, and yet all the separate blood elements had taken part in the cholesterine increase. In a physiological hyper-cholesterinemia, then, all the separate blood constituents take part in the general increase of cholesterine. In a pathological hyper-cholesterinemia, the separate blood constituents take a similar part, and it is reasonable to suppose that the same holds true in pathological hypo-cholesterinemia. The percentage relationship in the cholesterine content of the separate blood elements remains practically constant, both in hyper- and hypo-cholesterinemia. It follows, then, that either whole blood or serum alone can be used in quantitative cholesterine estimations.

CHRONIC NEPHRITIS

The study of cholesterinemia in relation to chronic nephritis is an exceedingly interesting one, presenting as it does those problems over which many authors have disagreed, especially the role played by the adrenal glands.

Although the kidneys do not assume the function of excreting

Curve No. 4.



Composite Temperature and Cholesterine Curves in Six Cases of Scarlet Fever.

Curve IV. Note the absence of any decided "cholesterine reaction." The disease was of insufficient duration to markedly influence the cholesterinemia. Practically no secondary hyper-cholesterinemia. Intensity and duration of exanthemata had no influence.

lipoids, it has been shown (6) that in nephritis, particularly in those cases accompanied by œdema, the measure of total lipoids in the blood is exceedingly high. It is quite generally admitted that a hyper-cholesterinemia is only one evidence of a general lipoidemia, and a hyper-cholesterinemia in chronic nephritis has been found by most investigators. My series of fourteen cases presented not only various clinical pictures but also various types in the severity of the kidney lesion and gave figures ranging from 0.933 to 2.38 grams of cholesterine per 1,000 c.c. of serum. The highest figures obtained, 2.31, 2.16, 2.10, and 2.38, were in cases largely, if not entirely, primarily nephritic, and despite the fact that in each of these four cases just mentioned the albuminuria was high, I found no consistent relationship between hyper-cholesterinemia and albuminuria, as is apparently the case with cholesterinemia and the urea content of the blood (7).

In uræmic conditions, the measure of cholesterinemia is comparatively low and I entertain the belief that cholesterinemia is, in some way, of prognostic importance not only in cases of nephritis, but in all cases in which the cholesterine content of the blood is influenced by the disease. Retinitis is often the first sign of an impending or already present uræmic state, and it has been shown (8) that the retinal plaques are local deposits of cholesterine derived without doubt from the blood. Furthermore, cases of nephritis without retinitis show a higher cholesterine figure in the blood than cases with retinitis. It is questionable though, whether this retinal deposit of cholesterine is a specific finding in albuminuric retinitis. This same lipoid has been found deposited or present in cases of sarcoma, detachment and traumatism of the retina. Hyper-cholesterinemia does not, necessarily, explain the cause of the retinitis, for frequently in cases of nephritis, with very high figures for the cholesterine content of the blood, no retinitis is found. Defaye (9) cites a case of chronic nephritis in which the first examination showed 1.42 grams per 1,000 c.c. Subsequent examinations during the course of the disease showed 2.10 and 3.72 grams per 1,000 c.c. and after the development of uræmia, which terminated in death, the cholesterine content of the blood fell to 1.21 grams.

Atheroma of the aorta, xanthelasma, arterio-sclerosis, and biliary calculi have all been shown to be further evidences of local deposits of cholesterine (10, 11, 12). And in all these conditions a hyper-cholesterinemia is found.

While considering cholesterinemia in nephritis, interest is directed to the adrenal bodies which are regarded by some investigators as the synthetic source of cholesterine (13). Chauffard, who associates hyper-cholesterinemia with adrenalemia and increased activity of the adrenal glands, regards the cholesterine that has been found in the cortex of the adrenal bodies, as the synthetic source of cholesterine. In my series of cases, hyper-cholesterinemia and increased blood pressure were not always associated, and Widal also claims that in nephritis hyper-cholesterinemia and increased activity of the adrenals may exist independently of each other. Landau (14) gives evidence to show that the cholesterine found in the cortex of the adrenals is to be looked upon as a deposit (retention) rather than a proof of its synthetic source. A functional insufficiency of these ductless glands must not be regarded as the cause of a hypo-cholesterinemia. Only recently I had the opportunity of examining the blood of an undoubted case of Addison's disease, and I obtained a normal cholesterine figure.

It can be safely said then, that in chronic nephritis a hyper-cholesterinemia is usually present, especially in cases of contracted kidneys; and the measure of this hyper-cholesterinemia is largely dependent upon the severity of the kidney lesion, in so far as it affects the general condition of the patient, and upon the presence or absence of such clinical factors as influence the cholesterinemia.

ARTERIO-SCLEROSIS

The deposit of cholesterine in the blood vessel walls—it is a deposit rather than an infiltration—is the primary step in the pathology of arterio-sclerosis. In thirteen cases of arterio-sclerosis the figures for the cholesterine content of the blood ranged from 0.75 2.16 grams per 1,000 c.c. and the amount found seemed to be dependent upon the duration of the pathological process. In the progressive stage of arterio-sclerosis, a hyper-cholesterinemia is found. In those cases with high blood pressure in which the sclerotic changes in the arterial walls had reached their maximum, I found no increase in the cholesterine of the blood; in some instances, in fact, a hypo-cholesterinemia. In two cases of apoplexy with arterio-sclerosis the etiological factor in both cases, I failed to find a hyper-cholesterinemia.

DIABETES MELLITUS

A hyper-cholesterinemia is the rule in diabetes. My series of six cases includes both mild and severe cases and the cholesterine content of the blood ranged from 1.28 to 2.91 grams per 1,000 c.c. The severity of the condition and the measure of glycosuria seem to influence the cholesterinemia, but I have insufficient evidence to show that any definite and consistent relationship exists between cholesterinemia and glycosuria. The figure 2.91 was obtained in a case in which the excretion of sugar in the urine amounted to 8 per cent. and in which the acidosis caused death soon after the blood was withdrawn for examination, while the figure 1.28 was obtained in a case in which the glycosuria amounted to 0.6 per cent. and in which no acidosis was present. The results of Bürger and Beumer (15) verify my findings. The hyper-cholesterinemia of diabetes is probably intimately associated with the marked metabolic disturbances which characterize the disease.

ACUTE INFECTIOUS DISEASES

Among acute infectious diseases, I examined the blood of cases of typhoid fever, scarlet fever, erysipelas, pneumonia and pleuritis. Of all clinical phenomena associated with these diseases I found none that so uniformly and materially influenced the cholesterine content of the blood as temperature. A rise in temperature sufficiently high to be termed fever (and enduring for a few days or more) is always accompanied by a fall in the cholesterine content of the blood, and this fall seems to be in direct relation to the intensity of the fever. So long as the temperature remains high so long does the cholesterine content of the blood remain low. If the temperature falls by crisis, the cholesterine increases correspondingly abruptly; if the temperature falls by lysis, the increase in cholesterine is correspondingly slow. Transitory rises in temperature do not necessarily lower the cholesterine content of the blood; fever must endure for several days before it influences the cholesterinemia. The cases of scarlet fever which came under my observation, exhibiting a short lived hyperpyrexia, did not show a decided hypo-cholesterinemia, as was always found in cases of prolonged fever, such as typhoid.

In typhoid fever I found a decided hypo-cholesterinemia during the fever period; a slow return to normal as the temperature approaches normal; a decided secondary hyper-cholesterinemia during convalescence, which may continue for weeks. The temperature and the general condition of the patient seem to be the chief influencing factors.

In pneumonia, I found a hypo-cholesterinemia during the fever period; a rapid return to normal or a hyper-cholesterinemia after the crisis and a secondary hyper-cholesterinemia of short duration if it presents itself at all. In slowly resolving cases the hypocholesterinemia is of longer duration. Here too temperature and the general condition of the patient are the chief influencing factors.

In scarlet fever the cholesterine reaction is slight, if it results at all, and is of short duration. There is no secondary hypercholesterinemia.

The results I obtained in cases of typhoid fever, pneumonia and scarlet fever, bear out those obtained by Chauffard, Laroche and Grigaut (16).

It has been assumed by some that cholesterine, especially in acute infectious diseases, plays a rôle analogous to an antitoxin. It is of interest to note in this connection that, after the critical stage of the disease when the combat between the body and the invading organism has been favorably decided, the cholesterine

3. The cholesterine content of the human blood varies not only in different pathological conditions but at different times during the course of the same disease.

4. Temperature and the general condition of the individual influence the cholesterinemia at all times.

5. Hyper-cholesterinemia is the rule in nephritis, diabetes and arterio-sclerosis.

6. Fever, cachexia, and debility produce a hypo-chloresterinemia.

7. A diet rich in cholesterine increases the amount in the blood.

8. Both in hypo- and hyper-cholesterinemia, all the separate blood constituents take part in the fall or increase and to about the same degree.

9. Cholesterinemia seems to be of prognostic importance in some diseases and an indicator of their severity. Researches in cholesterinemia are still in their infancy. Much work is necessary before the many problems this lipoid presents in its relation to the organism, both under normal and pathological conditions, can be solved. We have yet to determine more exactly, above all, its origin and destiny, the physiology and pathology of the quantitative variations in the blood, the significance of these variations, and the possible rôle cholesterine can play in therapy. content of the blood increased far in excess of the normal, thus simulating the over-production of antibodies on the part of the body during the same stage of the disease. And this secondary hyper-cholesterinemia, as I have termed it, seems to be dependent upon the demands made upon the organism during the course of the disease. On the other hand, before death caused by a disease, which under other circumstances would show a decided hyper-cholesterinemia, as in chronic nephritis, the cholesterine content of the blood diminishes materially. Can then a prognostic importance be attributed to the measure of cholesterinemia in certain diseases? And does the cholesterinemia in a case of disease indicate its severity? A careful review of the cases which came under my observation, and their clinical status at the time the re-

spective bloods were taken for examination lead me to believe, as in the case of nephritis, that these questions can be answered in the affirmative.

Cholesterine has been shown to exert other antitoxic properties. Phisalix in 1897 showed it to be antitoxic to the venom of a viper. According to Keys and Sachs, it is antitoxic to the venom of a cobra (see 17–9); Kemper and Schpilewski (17) claim it neutralizes the toxin of sausage poisoning, and Gerard and Lemvine (18) have shown that it attenuates tuberculin. Noguchi, Landsteiner, Eisler, Müller, Iscovesco and others have further shown that the hæmolytic action of serum is lowered by cholesterine. Whether it is antitoxic to tetanus is still questionable (19). Based on these properties, cholesterine has been given in the treatment of tuberculosis and the various anæmias (20, 21, 22).

In my original German paper, I present my findings in a considerable number of miscellaneous conditions.

SUMMARY

1. Accurate and applicable cholesterine extraction methods are available. Colorimetric quantitative determinations are reliable for clinical observations.

2. Either whole blood or serum alone can be used in making the determinations.

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CULTURAL STUDIES OF THE VIRUS OF RABIES

HIDEYO NOGUCHI

Dr. Noguchi described the methods which he had used in cultural studies upon the virus of rabies, and reported upon the results of animal inoculation of culture preparations. Histological findings were also described and illustrated with a series of lantern slides.

A complete report of Dr. Noguchi's studies will be published elsewhere.

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[1] The papers published in this number were read at a joint meeting of the New York Pathological Society and the Society for Serology and Hematology held at the New York Academy of Medicine, December 10, 1913.

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DR. A. B. WADSWORTH, *President*

A CASE OF CONGENITAL CYSTIC KIDNEY

B. M. VANCE

(Pathological Department, Bellevue Hospital, Charles Norris, Director)

Many cases of congenital cystic kidney have been described, but at the present time the pathogenesis of this condition has not been definitely determined. Some of the older observers attempted to discover the origin of the cysts in the interstitial tissue between the kidney tubules. Examples of such theories are those of John Simon, who regarded the cysts as the outcome of a much enlarged epithelial cell which escaped from a ruptured tubule into the interstitial tissue of the kidney, and had there undergone hydropic degeneration, and Luschka, who looked upon the cysts as the result of apoplectic hæmorrhages into the interstitial tissues. At the present time the prevailing conception is that the cysts of congenital cystic kidneys take their origin from the kidney tubules proper, and this has given rise to three different theories to explain their genesis.

1. The first theory presupposes an inflammatory process in the kidney during fœtal life, causing obstruction of the tubules, retention of secretion and subsequent cystic formation. Some observers have claimed that the stoppage was due to a fibrinous exudate in the lumen of the tubules; others, that it was due to the epithelial plugs caused by a desquamative nephritis. One of Virchow's earlier theories was that the obstruction resulted from uric acid concrements which had formed in the straight tubules. Later, he considered that the retention arose from an inflammation of the papillæ, and a subsequent connective tissue proliferation in this region which caused an atresia of the tubules. The causative factor of this inflammation was some toxic substance which was transmitted by the mother to the fœtus. Thorn was another adherent of the inflammatory theory, but differed from Virchow by making the pelvis of the kidney the starting point of the inflammation which spread from this point to the papillæ. Arnold and Leichtenstern regarded the inflammation as a chronic interstitial nephritis which snared off the tubules by fibrous tissue proliferation. Leichtenstern suggested the possibility that the inflammation could have originated from the tissue around the arteriolæ rectæ.

2. Other observers, for example Nauwerck and Hufschmid, and v. Kahlden regarded the cysts as neoplastic in nature and arising from the epithelium of the urinary tubules by proliferation. They considered that the process was identical to the formation of the adenocystoma of the ovary. Nauwerck and Hufsschmid believe that the cystic formation begins as a colloidal degeneration of the epithelium while later the fluid of the cysts comes as a transudate from the blood vessels of the cyst wall. v. Kahlden regarded this colloid change and transudation as a

negligible factor, and declared that the cystic contents were due to the secretory activity of the glomeruli which still remained in the cysts.

3. The third theory described the origin of the cysts in developmental disturbances in the fœtal kidney. The exponents of this theory were Ribbert, Herxheimer, Meyer, Busse and others. They based their argument on the fact that the kidney arises in embryonic life by two anlage, one for the ureter, pelvis and the straight tubules, and another for the glomeruli and coiled tubules, in direct opposition to the theory of Gerhardt, who considered that the kidney and ureters arose by one anlage, namely, the renal sprout. In the course of development in fœtal life, these two systems fuse and form the ordinary adult form of renal tubule. The cystic kidneys occur when for some unknown reason there has been a failure of union between these two systems causing the formation of a closed sac composed of the Bowman's capsule, the glomerulus and the coiled tubules. Retention of secretion and cyst formation thus results. Beitzke described a case where apparently the cysts arose from the anlage of the straight tubules in the medulla.

It is difficult to say which one of these three theories is the correct one, though at the present time, the theory of developmental disturbances is considered with more favor than the theory of inflammation in embryonic life, or the neoplastic theory. The two last mentioned theories cannot be excluded altogether, though the theory of developmental defect offers the most reasonable explanation for the formation of cystic kidneys, and has the most evidence in its favor. Herxheimer enumerates four facts in support of this theory.

First, that the disease often occurs in families. In cases described by Steiner, Höhne, Jacob and Davidsohn and others, individuals of the same family, mother and daughter, father and son, or two sisters, were found to possess cystic kidneys.

A second point is the very common finding in cystic kidneys of tissues which are as a rule only found in the kidney in embryonic life. Busse, Beitzke and others have described smooth muscle fibers in the wall of cysts and Busse has described cartilage in a cystic kidney.

The third fact is the combination of cystic kidneys with cysts in other organs, especially in the liver, and quite rarely in the pancreas, spleen, plexus, choroideus, brain, uterus, thymus, etc.

The fourth and last point is the combination of cystic kidneys with developmental defects elsewhere, such as hydrocephalus, encephalocele, polydactylism, cleft palate, hare lip, and malformations of the genito-urinary tract of a very severe grade. Examples of these are phimoses, malformations of uterus and prostate, atresia of the ureters, and so on. This congenital atresia of the ureters was supposed by some to be a cause of the cystic kidneys, until Peterson and others proved conclusively that an experimentally produced impenetrable ureter always resulted in a hydronephrosis, never in a cystic kidney. As a general rule, these severe accompanying developmental defects are usually found in cases of cystic kidneys discovered in very young infants.

Most congenital cystic kidneys have been found either in newborn infants, or in adults over thirty years of age, though a few cases have been described which lie between these two extremes. The two classes of cases seem to be distinct at first glance, but as Busse declares, the pathogenesis is the same and the cystic kidneys in adults merely show a less extensive change than those of infants, and possess a larger amount of functionating parenchyma which will carry them on for a longer period of existence. The cystic kidneys of new born infants are usually bilateral and morphologically present a great variety of forms, either a single kidney formed of one to six very large cysts or two kidneys composed of a large number of very small cysts. The amount of functionating parenchyma is usually negligible, and, as stated above, there is as a rule some other severe developmental defects of the genito-urinary tract present.

The involvement in adults is as a rule bilateral, though a large number of cases have been described of unilateral cystic kidney occurring mostly on the left side. The second case of Herxheimer is one type of such a growth, where the left kidney possessed an obliterated pelvis and consisted only of six large cysts, while the right kidney appeared normal. The usual form of adult cystic kidney, however, is a very large nodular kidney, which may weigh 3,000 grams, and be larger than a child's head, which is composed of numerous small bluish cysts projecting on its surface, varying in size from a cherry stone to a man's fist, and containing between them normal functionating kidney parenchyma. The cysts are closed, as a rule, with connective tissue walls in which some (Busse, Beitzke) have described smooth muscle fibers, and lined by flat or cubical epithelium. They are usually distributed in the cortical portion of the parenchyma, and less so in the medulla. The contents are usually a clear serous fluid, of a high albuminous content, containing a few urinary constituents, and much sediment and crystals of various kinds among which are the "rosette" forms, described by Beckman, Höhne and Jacob and Davidsohn. These will be discussed later. Less often the cyst fluid is bloody or brown from hæmorrhages, or purulent from infection in cases of pyemia.

The cases of congenital cystic kidneys show a very varied clinical course. The clinical course of cystic kidneys in new born infants is not very striking, as the patients die as a rule in a few months after birth. In adults, however, the clinical picture is interesting in the light of the pathological findings. Albarran and Imbert have recognized three main clinical types of congenital cystic kidneys in adults. In the first type, the patient runs a symptomless course until he shows uremic symptoms and dies. In the second, he suffers from symptoms of chronic interstitial nephritis, such as digestive disturbances, headache, polyuria, etc., until he finally ends his life in uremic coma and convulsions. In the third, the patient complains of pain, in one lumbar region, hematuria, renal colic, and other symptoms of the so-called "surgical kidney."

According to Sieber, the presence of cysts which encroach on and cause damage to the normal kidney parenchyma, has apparently the same effect on the organ that the connective tissue has, in a chronic interstitial nephritis. The heart may be quite hypertrophied, and the vessels may be sclerotic. Hæmorrhages, too, are prone to occur by rupture of a vessel, especially in the brain.

The urine of these cases may be absolutely normal in quality and quantity, and may also resemble the urine described as typical of a chronic interstitial nephritis, showing large daily excretion, low specific gravity 1,005–1,015, clear amber color, and containing a trace of albumen and a few hyaline casts. At intermittent intervals, there may be found in the urine the typical "rosette" forms described by Höhne, and Jacob and Davidsohn, which show microscopically a clear nucleus surrounded by radiating streaks. Höhne looks on these as composed of colloid material. Jacob and Davidsohn regard them as pathognomonic of cystic kidneys and ascribe their presence in the urine to the rupture of a cyst into the pelvis of the kidney. They also claim that the puncture of one of these cysts through the body wall during life and the discovery of the "rosette" forms in the fluid will help establish the diagnosis. Occasionally blood occurs in the urine, whenever a hæmorrhage has taken place in a cyst, and the cyst has ruptured into the pelvic cavity. In this case, the passage of blood down the ureter is liable to give rise to pains of renal colic. Pus may appear in the urine in cases of cystic kidney whenever the cyst becomes purulent from a pyemic infection and rupture into the pelvis. Such a complication is attended as a rule by severe pain and tenderness in the kidney region, and a typical septic temperature.

The physical sign given by the cystic kidneys is usually the discovery of a large, irregular cystic mass, rarely on both sides, but usually on one side, even in cases where both kidneys are involved. This large mass is usually found to be quite movable and often misplaced, even as low as the pelvis. Its size may be such as to cause pressure symptoms in the various abdominal organs, such as constipation from pressure on the intestines, or a tendency to abortion in pregnant women.

The diagnosis of this condition is quite difficult because the symptoms and physical signs are not absolutely characteristic, while some attending process may overshadow the disease. In a few cases the diagnosis was correctly made during life, as in the case of Osler, who diagnosed the condition because it had been found in other members of the same family. The presence of palpable cysts in the liver will help out in the diagnosis, but this condition is not at all common. As a general rule, cases of congenital cystic kidneys in adults have been diagnosed as other kidney conditions, or disease which caused enlargement of the ovary, spleen, etc.

Congenital cystic kidneys are associated in about 19 % of the cases, according to Moschcowitz, with cysts in the liver. Like the cysts in the kidneys, these vary much in size, ranging from microscopic cysts to cysts so large as a child's head. The larger ones are usually thin walled and contain a thin serous (rarely turbid or hæmorrhagic) fluid which gives the cyst a bluish appearance. Microscopically the smaller cysts can be easily seen enclosed in the capsule of Glisson and lined by a cubical epithelium which resembles the bile duct epithelium very markedly. Some cysts appear to have a glandular structure. In addition to the cysts, many small aberrant bile ducts are found here and there running in the capsule of Glisson. Neither the cysts nor the aberrant bile ducts appear to have any connection with the liver cells.

The theories offered to explain the genesis of the liver cysts are just as many and as varied as the ones that have been put forward to explain the genesis of cystic kidneys. Among them must be mentioned the retention theory, which regarded the cysts as dilated bile ducts shut off from the general biliary system by inflammatory lesions in cirrhotic livers, and the neoplastic theory, that the cysts were true tumor formations. The most probable theory, however, is the theory of developmental disturbance which considers the liver cysts as arising from aberrant bile ducts which have become separated from the bile duct system in embryonic life. There are many points which uphold this theory, among which are the frequent association of cystic livers with cystic kidneys and other developmental anomalies, the absence of bile in the liver cysts, and the presence of aberrant intracinous bile ducts in cystic livers. Moschcowitz has an excellent review of the subject in the *American Journal of Medical Sciences,* vol. 131, 1906.

Clinically cystic livers are of little importance, except in old individuals, in whom the cysts have reached enormous proportions. The symptoms are usually those of an advanced case of liver cirrhosis, such as ascites, gastro-intestinal catarrh, intestinal hæmorrhages, etc. When the cysts have reached an enormous size, they may be palpable on the liver through the abdominal wall.

CASE REPORT

Mary W., aged 30, was admitted to the medical wards of the third division of Bellevue Hospital in the service of Dr. Carlisle on August 31, 1913. The family history, and previous medical history, were negligible. Her present illness was of two months' duration, during which time she suffered from a cough with expectoration and general malaise, and lost considerable weight.

Physical examination showed a very emaciated young woman, quite anæmic and apparently very ill. Physical signs in chest revealed the presence of a pulmonary tuberculosis. The heart was very rapid and irregular, but otherwise showed nothing unusual. In the abdomen, a large irregular mass was palpable, extending from the free border of the ribs on the right side about 5 by 4 inches, of firm consistency, not connected with the liver, and movable six inches in any direction. It was supposed to be an enlarged kidney.

The urine examination showed a light amber turbid fluid, specific gravity 1,015, acid in reaction, containing a trace of albumen, no sugar, a few hyaline casts, a few leukocytes, and a small amount of amorphous sediment. No tubercle bacilli were found in the urine. There were, however, tubercle bacilli present in the sputum in some numbers. The blood count showed leukocytes 11,000; polymorphonuclears, 72 per cent.; lymphocytes, 22 per cent.; mononuclears, 6 per cent.

Clinical Diagnosis: Pulmonary tuberculosis, tuberculosis of right kidney.

The body was autopsied on September 8, 1913, eight hours after death. The body was that of a female, 174 cm. in length, very emaciated and quite anæmic, as shown by the sallow skin and pearly white scleræ of the eyes.

The peritoneal cavity contained about 700 c.c. of clear serous fluid. The lungs showed old fibrous adhesions around their apices, but no fluid in either pleural cavity. There was a large number of discrete shot-like bodies of yellowish white color, apparently miliary tubercles, found throughout both lungs. In the apex of the left lung were three walled-off cavities containing a purulent exudate and varying in size from 1½ to 2 cm. in diameter.

The heart showed nothing except a slight cloudy degeneration.

The gastro-intestinal tract contained two small tuberculous ulcers, one in the ileum, and the other in the cæcum. The pancreas was apparently normal. The spleen was atrophic. The liver was not abnormal on microscopic examination, and showed only a slight parenchymatous degeneration. The adrenals were apparently normal.

The kidneys on external examination were composed of small cysts and had a mulberry-like appearance (see photograph of gross specimen, figure 1). The cysts appeared bluish, varied in size from 0.5 cm. to 5 cm. in diameter, and were filled with a clear serous fluid rich in albumen. They were thin walled, and were scattered throughout the kidney, predominating mostly in the cortex. The cysts were discrete cavities, in no way connected with each other or the pelvis of the kidney. On cut section between the cysts, could be seen areas of normal kidney tissue, irregularly arranged. The kidneys were somewhat enlarged, the right measuring 15 by 10 by 5 cm., while the left measured 15 by 8 by 5 cm., but they had retained to a great extent their characteristic kidney outlines.

The ureters were short, about 27 cm. long, and when viewed externally, seemed to enter the kidney hilum without forming a pelvis. When the kidney was sectioned, however, the pelvis could be seen well within the hilum where it sent off long infundibula which divided into many smaller infundibula to join with the kidney substance. The infundibula were, perhaps, longer than are usually seen in normal kidneys. The blood vessels divided into three or four separate branches before they entered the kidney hilum. The bladder was normal. The uterus and adnexa showed nothing unusual, except a small subserous fibromyoma on the anterior surface of the uterus and a small cyst of the paroöphoron near the left ovary.

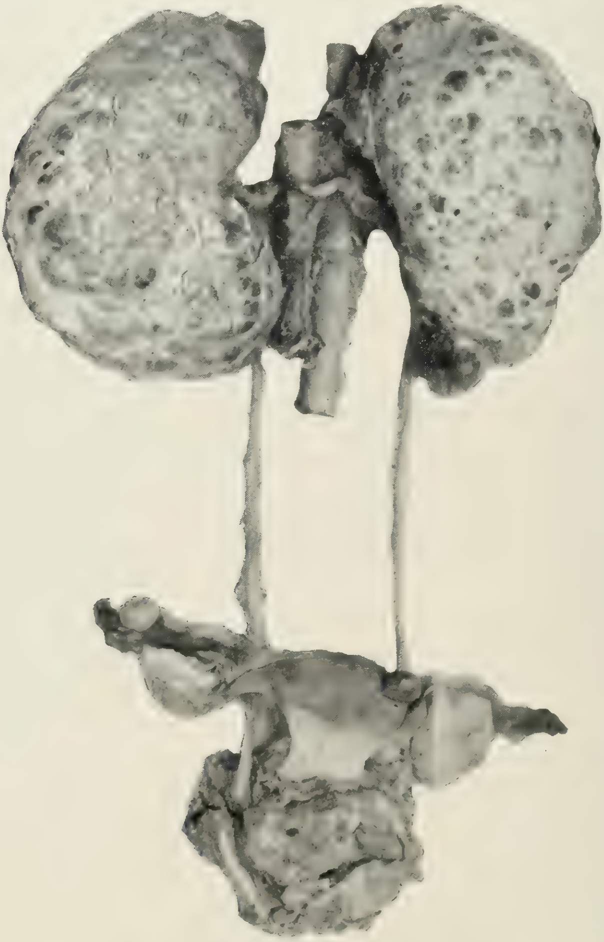

FIG. 1. CONGENITAL CYSTIC KIDNEYS.

On microscopic examination, the liver showed the presence of microscopic cysts which were placed in Glisson's capsules and surrounded by connective tissue. The cysts were lined by cylindrical and cubical epithelium very similar to the epithelium of smaller bile ducts. In some cases there was a single large cyst, or a number of smaller cysts present, in the same portion of the capsule, which gave the picture an adenomatous appearance. A number of small aberrant bile ducts were also seen in different portions of the capsule. These aberrant ducts and the cysts apparently did not connect with the bile capillaries, as was ascertained by examination of serial sections cut in celloidin. However, as the larger bile ducts were not injected before hardening, such evidence is not wholly conclusive. The cyst contents appeared to be a mucoid substance which took a light eosin stain. No bile was found in the cysts.

The kidney showed on section large cystic spaces lined by flat cells, much like blood vessel endothelium. The walls of the cysts showed a very slight connective tissue framework in which no smooth muscle fibers could be demonstrated by Van Gieson's stain. There were no papillomatous growths in the cysts. Between the cysts, the kidney tubules and glomeruli appeared normal, save for a marked grade of chronic interstitial nephritis.

The sections of the other organs showed nothing unusual.

The contents of the individual cysts were unfortunately not examined microscopically, nor was any extensive chemical examination made of the fluid.

CONCLUSIONS.

It seems most probable that the theory of developmental disturbance would best explain the presence of cysts in the liver and kidney in this case. The association of these two conditions in the same individual, the widespread kidney involvement and the presence of cysts and aberrant bile ducts in the liver, all point to an origin in embryonic life. In this case the development of the condition was apparently gradual, as is the case in adult forms of cystic kidneys, and enough kidney parenchyma was apparently left to tide the patient over childhood and adolescence.

For literature see

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Discussion:

DR. NORTHRUP: I have encountered many such cases in children and not one of them gave any symptoms until death. I have noted that when one finds one developmental defect it is usually the case that there are three or four more, such as hare lip, spina bifida or club foot, for example. In this case there seemed to be cysts all through the kidney and liver and other defects.

DR. LIBMAN: It has struck me that the occurrence of congenital defects of the heart with congenital cystic disease of the kidneys may not always simply indicate a tendency toward the development of defects in more than one part of the body at the same time.

Some years ago I saw a child with small cystic kidneys in which was present a great deal of fibrosis. The left ventricle was somewhat hypertrophied and ductus arteriosus was open. It struck me at the time that the ductus might have been kept open by the high blood pressure in the left ventricle due to the presence of the diseased kidneys.

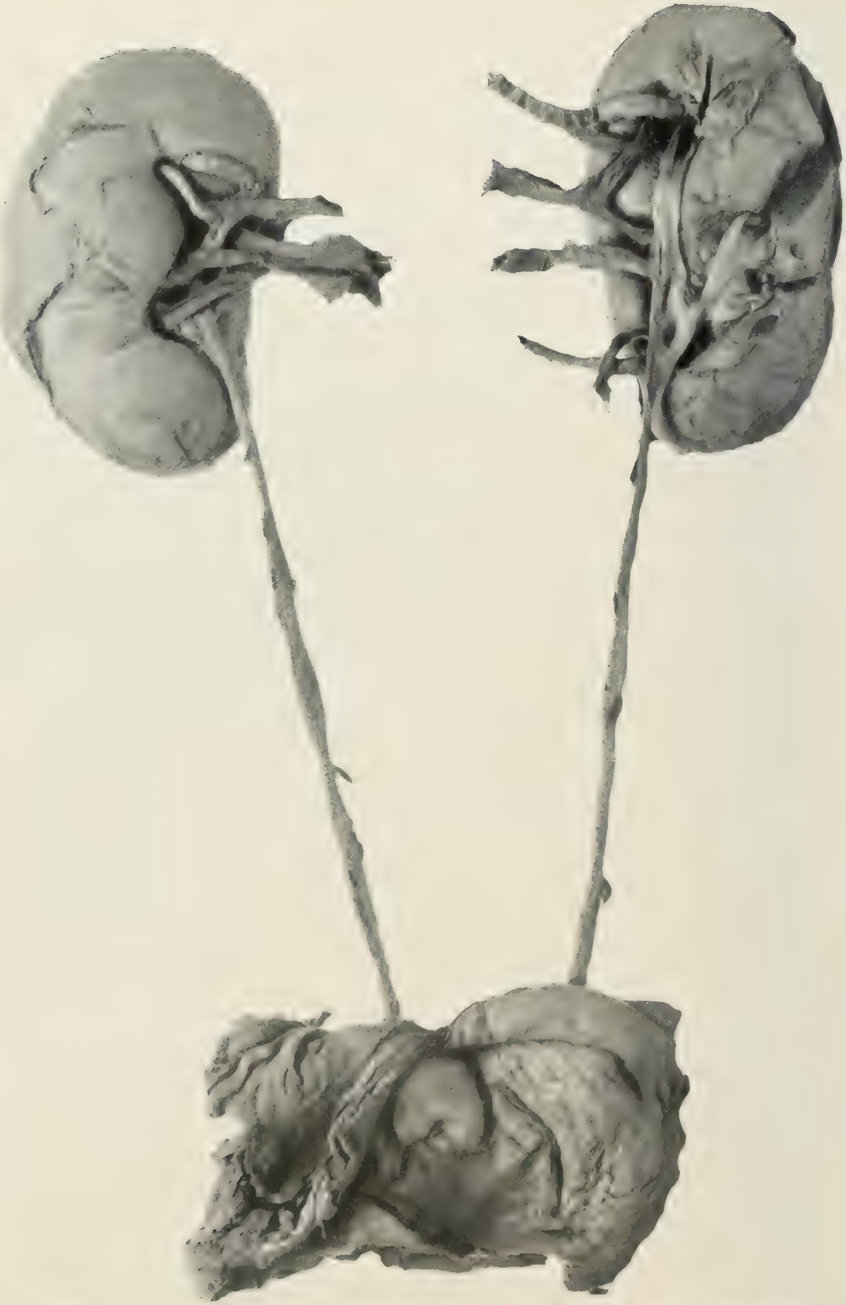
A CASE OF CONGENITAL MALFORMATION OF THE URETERS AND BLOOD VESSELS OF BOTH KIDNEYS

B. M. VANCE

(Pathological Department, Bellevue Hospital, Charles Norris, Director)

The patient, in whom this anomaly was found quite by accident, was a man of 52 years of age, who showed as his principal lesions a carcinoma of the gall bladder, secondarily involving the liver, pulmonary œdema, jaundice and ascites. The vertebral arteries at the base of the brain were anomalous, as the large right vertebral carried on almost the entire circulation in this region while the left was represented by a very minute thread-like branch. The kidneys, aside from the anomaly present, showed nothing more unusual than a rather deep grade of jaundice, some chronic interstitial nephritis and parenchymatous degeneration. The left kidney was about the normal size; its dimensions were 12 cm. by 7 cm. by 4½ cm. It did not have the characteristic kidney shape, but was rectangular in outline with rounded edges. The fœtal lobulations were not well marked on the surface. The kidney possessed no true hilum, but received its blood vessels and ureters on its anterior surface. The ureteral supply was represented by four ducts which left the kidney substance at various points on its anterior surface and ran externally to meet in a common pelvis situated about 3 cm. from the lower pole and 1 cm. from the inner edge. From this point the pelvis was drained by a single ureter which ran 24 cm. before it emptied by a single opening into the bladder. The longest of the four ducts emerged from the kidney substance about the middle of the upper pole of the kidney and ran about 7 cm. before it joined the other three. One of these was situated in the middle portion of the kidney close to the inner border, while the other two ran from the inner and outer portions of the lower pole respectively. The last named duct was dissected out and found to widen into a miniature pelvis which further subdivided into two calyces which drained a papilla apiece. While the other ducts were not fully dissected out, it can be assumed that they all widened into similar pelves, which drained a definite division of the kidney parenchyma. This division could be made out on section of the kidney substance.

The arterial supply was by three parallel branches from the abdominal aorta which entered the kidney substance on its anterior surface. The upper branch entered around the pelves in the upper pole of the kidney, sending a sub-branch in front of and another behind the duct. The middle and lower arteries ran to the pelves in the middle and lower mesial portions of the kidney, sending their branches into the kidney substance in precisely the same way. The venous supply was by two branches of the in-



Blood Vessels and Ureters of Kidneys Showing Developmental Anomaly.

ferior vena cava, a larger upper branch which was formed by union of two tributaries from the upper pelvis and middle mesial pelvis respectively, and a smaller lower branch which drained the lower mesial pelvis. (See accompanying photograph.)

The right kidney had a more characteristic kidney shape, and was 12 cm. by 7½ cm. by 4½ cm. in its dimensions. It possessed a rather large hilum, about 5 cm. in length and situated in the middle portion of the kidney on its anterior surface about 1½ cm. from the inner border. The ureter of the right kidney resembled those of the left as it was found by four separate ducts which joined each other just as they emerged from the hilum. There was only one renal artery and vein, branches of the abdominal aorta and inferior vena cava respectively, which divided into sub-branches to enter the hilum in front of and behind the kidney pelvis.

The bladder, prostate, testicle and other pelvic organs were apparently normal. The adrenals were normal, and were apparently placed in the usual situation in relation to the kidneys. Unfortunately, the exact position of the kidneys was not ascertained at autopsy, except to note that they were situated well above the pelvic brim. Their position must, however, have been much lower than the usual location, because of the short ureters which were about six centimeters below the normal ureteral length.

The only other case in the literature which resembles the above description is the one reported by Richmond. From each kidney there were given off four separate infundibula which met at a point four inches below the kidney to form a common pelvis. This was emptied by a single ureter which opened into the bladder in the normal fashion. The description of the case was very meager and many points of importance were not mentioned.

There have been other cases reported, however, where the kidney on one side has shown a marked division of the pelvis into three or four separate infundibula. Such were the cases described by McMurrich and Grüber. Such kidneys were usually placed low in the body cavity or showed some other type of congenital displacement. Oscar Meyer (in *Ziegler's Beitrage*, Vol. 44) showed diagrams of horseshoe kidneys and congenitally displaced kidneys with pelves divided into three or four infundibula, though no mention is made of these points in the text.

A consideration of the embryology of the kidney is necessary for the full understanding of this anomaly of ureteral division. Pohlman outlines the essential points as follows:

1. The renal bud lies dorsally on the Wolffian duct after the duct has reached the cloaca, and it is a short distance from its entrance.

2. The two buds grow dorsally lying close together, each kidney being capped with a mass of renal mesenchyme.

3. Each bud divides into an upper and lower sprout at some distance from the Wolffian duct.

4. The kidney wanders upward from a position in front of the second sacral vertebra, rotates at the mid-lumbar region and finally reaches its normal height by the end of the second month.

5. It becomes vascularized after it has reached its normal height.

6. The ureter changes its position on the Wolffian duct from dorsal to lateral, and comes to empty distinct from it.

7. The ureter loses its relation to the duct entirely and opens higher and more lateral.

Pohlman assumes that the kidney is formed from a double anlage consisting of the renal mesenchyme, which gives rise to the glomeruli and coiled tubules and the renal bud from the Wolffian duct which gives rise to the ureter, pelvis and straight tubules. The two primary divisions of each renal bud are represented in the adult by the upper and lower divisions of the pelvis, which is more or less marked in adult kidneys. When the renal bud has divided earlier than the usual time, we get various degrees of separation of these two primary divisions, such as are represented by the split pelvis, incomplete ureteral division, and the double ureter. The two primary sprouts furthermore may subdivide into secondary branches, and we may get three or four subdivisions of the kidney, pelvis, and ureter depending on whether one or both primary divisions participate in the subdivision. Like the primary division, these subdivisions all take place in the same plane, so that the pelves would lie directly one above the other.

These few points suggest that the anomaly in the case described above can best be explained by an early division and subdivision of the renal bud into four definite branches which were all capped by the primary mass of renal mesenchyme. Evidently the division did not follow Pohlman's rule and take place in one plane.

The short length of the ureters, and the anterior situation of the pelves and blood vessels, indicate that the kidneys reached their permanent position in the body cavity in embryonic life much lower than the usual level, and before they had completely rotated inward on their longitudinal axes. The anomalous distribution of the blood supply to the left kidney is another point in favor of this assumption, for the three parallel renal arteries have a strong resemblance to and are perhaps the remains of the parallel branches of the abdominal aorta which supply the Wolffian body. In the normal course of events, these arteries atrophy when the kidney reaches its normal level and becomes vascularized. Whether they have persisted or not in this case, is impossible to say, though Anitschkow mentions cases of dystopia of the kidney whose arterial supply he interprets as a persistence of the mesonephric arteries, because of their parallel arrangement and position on the abdominal aorta.

This anomaly, like many others of the ureters and kidney, is interesting only from an anatomical point of view and is hardly important in a pathological sense. There were no symptoms in this case referable to the kidneys that had any connection with the malformation, and the patient died as the result of a carcinoma of the gall bladder.

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VEGETATIVE ENDOCARDITIS OF THE PULMONARY VALVE; THROMBOSIS OF ONE OF THE BRANCHES OF THE RIGHT PULMONARY ARTERY; EMBOLUS OF THE LEFT PULMONARY ARTERY; PATENT VENTRICULAR SEPTUM

ELI MOSCHCOWITZ, M.D.

Clinical History. Rosie D., 29 years old, admitted January 19, 1913, to the Beth Israel Hospital, service of Dr. Reinthaler. Died September 26, 1913. Married eight years; four children, three still-births. Pneumonia three years ago; had typhoid in childhood. Has never had any of the other infectious diseases. Has had a "weak heart" since her fifteenth year. She has had frequent attacks of cardiac palpitation and dyspnœa. Has occasionally edema of the legs. The *present history* is of four months duration. She complained of weakness, anorexia, edema of the legs, frequent headaches, dizziness, nausea, frequent vomiting and severe cough. The cough was at first dry but is now accompanied by much expectoration. Has severe attacks of palpitation. Seven days ago suddenly developed fever, chills, cough accompanied by pain in the sides, bloody sputum and dyspnœa.

Physical Examination: (Only the noteworthy features will be mentioned.) There was marked prostration, dyspnœa and cyanosis. *Heart:* Apex beat in fourth interspace, 4½ inches to the left of the median line. The right border was 1½ inches to the right of the median line. Upper border was at the third rib. There is a soft systolic murmur at the apex transmitted to the axilla. The second sound at the aorta was replaced by a loud systolic murmur transmitted to the vessels of the neck. The *liver* and *spleen* were not enlarged. Sibilant and sonorous râles heard over both lungs. The *temperature* was of the septic type, low or normal in the morning, reaching 102° 104° in the evening. Very often the temperature was subnormal, reaching even as low as 95°. Throughout her stay in the hospital she had frequent chills, accompanied by a rise in fever. The *pulse* varied between 80 and 90 throughout her illness; occasionally it would run up to 100 or over. During the last forty-eight hours, the pulse averaged 110. Her stay in the hospital was characterized by the incidence of numerous infarcts in the lung, attended by the classical clinical and physical evidences. Her *spleen* slowly increased in size until in the terminal stages of her illness it could be felt about three fingers below the costal margin. She was subject to numerous petechiaæ which had a tendency to appear in crops. The *urine* was free from albumen until the beginning of August when a faint trace appeared. This albumen persisted until death. The cardiac signs did not appreciably change. She gradually became weaker. The pulse became more rapid and the day before she died she complained of severe pain in the abdomen. Her death was a slow one, edema of the lungs occurring many hours before exitus.

Seven blood cultures were taken, all of which were negative with the exception of one, taken May 20, which showed a growth of *Streptococcus viridans*.

Autopsy. (Only the important features will be mentioned.) *Right lung* is collapsed. The anterior margin of the middle lobe is the seat of a huge infarct. The entire remainder of the middle lobe is the seat of gray hepatization. *Left lung* is collapsed and very edematous. *Heart* is enlarged. Left ventricle is slightly hypertrophied, but shows no dilatation. The mitral valves show a little atheroma. Right ventricle: muscle is one cm. thick; cavity considerably dilated. The *pulmonary valves* are completely destroyed and at their site there are numerous large grey firm pedunculated vegetations, varying in size from a pea to a hazel-nut. These vegetations extend upward upon the wall of the pulmonary artery to its bifurcation, but in this situation are much smaller in size. These vegetations have contracted the communication between the pulmonary artery and the light ventricle to a narrow slit. At the terminal end of the right branch of the *pulmonary artery* there is a hard dense sessile thrombus occupying that branch of the artery that supplies the middle lobe of the lung. The terminal end of the left branch of the pulmonary artery is occupied by an embolus which completely fills the lumen. The embolus is the size of a pea and of the same character as the vegetations below.

There is a communication in the membranous portion of the intraventricular septum admitting a pencil. *Liver* is adherent to the diaphragm. It presents the typical "nutmeg" appearance. *Pancreas* is normal. *Spleen* is enormously enlarged, 9 × 6 × 4 inches, and the seat of numerous fresh and healing infarcts. Pulp soft and velvety. Malpighian bodies distinct. The *kidneys* are the seat of numerous small infarcts, many of which are healed. Parenchyma pale, cortex cloudy, markings distinct; cyanosis.

Anatomical Diagnosis: Subacute vegetative endocarditis of the pulmonary valve (*Streptococcus viridans* infection); embolus of the left branch of the pulmonary artery; thrombosis of the right branch of the pulmonary artery; infarct of the middle lobe of the right lung with secondary lobar pneumonia; hypertrophy of both ventricles with dilatation of the right ventricle and patent ventricular septum; edema of the lungs; chronic passive congestion of the liver with perihepatitis; hyperplasia and congestion of the spleen with multiple infarcts; parenchymatous nephritis with chronic passive congestion and multiple infarcts.

Dr. Moschcowitz thought the case worthy of report for the following reasons:

1. Vegetative endocarditis of the pulmonary valve is very unusual. Dr. Libman, whose experience in this disease has been exceptionally wide, states that he has seen but one other case.

2. The probability is strong that the endocarditis was engrafted on a congenital lesion. This is surmised from the history of early heart disease without the usual etiology, and the

presence of a pronounced congenital heart lesion in the form of an open ventricular septum.

3. A bilateral embolic lesion of both branches of the pulmonary artery is most unusual and as far as Dr. Moschcowitz was able to determine from a study of the literature is unique.

4. The case demonstrates that it is possible to have a complete plugging of a branch of the pulmonary artery without causing sudden death. A number of such cases have been reported by Hart, quoted in Osler's *System of Medicine* by R. C. Cabot.

5. The association of embolic evidences in the general circulation with a thrombotic lesion limited to the pulmonary artery. The open ventricular septum manifestly explains this.

THROMBOSIS OF AORTA AND BOTH ILIAC ARTERIES FOLLOWING STREPTOCOCCUS INFECTION OF THE UMBILICAL CORD

ELI MOSCHCOWITZ, M.D.

The organs presented were removed from an infant ten days old. The child was born in the Jewish Maternity Hospital after a normal labor. Within a day or two of birth the child began to run a septic fever. About twenty-four hours before the child died the lower extremities became cold, cyanotic and slightly edematous. The cord dropped off at the usual time, and healed kindly with a tough, dry scar. The autopsy showed a firm red thrombus in the abdominal aorta projecting upward in the shape of a thin cone for a distance of 2 cm. above the bifurcation of the iliacs. Both iliac arteries as far as they could be dissected were completely filled by a thrombus of the same character. The umbilical vein was dilated and filled with purulent coagula. Smears from these coagula showed many streptococci; these were also demonstrable in the wall of the umbilical vein.

Sepsis from the umbilical cord in the newly born may travel either by the umbilical vein or the umbilical arteries, or both.

The arterial mode of transmission is said to be more common than the venous in the proportion of two to one.

As far as the writer has been able to investigate, he has not found in medical literature a case of septic thrombosis of the aorta following umbilical infection.

Clinically the case is interesting as showing that a profound umbilical sepsis may exist without any external manifestation of sepsis of the cord.

TUMOR OF KIDNEY WITH UNUSUAL MICROSCOPIC FEATURES

ALEXANDER FRASER, M.D.

(Department of Pathology, University and Bellevue Hospital Medical College)

This tumor was removed from a patient in St. Vincent's Hospital by Dr. Geo. D. Stewart. The patient was a female, aged fifty. She came to the hospital complaining of a cold and showed no symptoms referable to the kidney. During the physical examination a large mass was discovered in the region of right kidney; an operation was performed and this tumor removed. I thought its presentation might be of interest, first on account of the variegated histological appearance which it presents, and secondly, from the standpoint of the etiology of renal hypernephroma.

The gross appearance suggests a tumor of renal origin. It is covered by a connective tissue and fatty capsule, apparently the original capsule of the kidney. The surface presents several large rounded lobes from which project, in places, smaller lobules, some of which are hemorrhagic in appearance. On section some of the large lobes are seen to be of a cystic character, that is, partly excavated and containing a gelatinous looking material.

It is the microscopic findings that are most interesting. Histologically the tumor shows every picture, as far as I know, that has ever been described in renal hypernephroma: (1) the ordinary picture in renal hypernephroma, the picture so similar to that of the adrenal cortex that it has given to this tumor its name and its older theory of origin, viz., its development from suprarenal rests; (2) tubular formation, the lining epithelium being of a lower cuboidal type and the lumina of the tubules filled with blood; (3) larger epithelial-lined spaces with papillary ingrowths of the walls; (4) areas of a spindle-cell sarcomatous type.

These four pictures have been described as occurring in renal hypernephromata, but in this tumor there is a fifth picture which has not been recorded in hypernephroma of the kidney, but which seems to me to be very similar to the characteristic picture seen in hypernephroma when it occurs in the adrenal organ itself. In this picture the cells are distinctly carcinomatous in type, of very variable size and appearance and among them are multinucleated giant cells of the myeloid type.

It would be interesting to have an expression of opinion as to the nature of the tumor and as to whether any of these histological appearances can throw any light on the problem of the origin of renal hypernephromata. The older Grawitzian theory that they are neoplasms of adrenal rests has been abandoned by the majority of later observers. Stoerk believed that they were of renal origin, from the convoluted tubules. Wilson and Willis on the strength of embryological facts conclude that they develop from Wolffian body "rests." English observers (Nicholson, Dunn and Glynn) believe that they have found evidence for the theory that they originate from cyst-adenomata of the kidney, occurring in connection with chronic interstitial nephritis. In eighty autopsies Dunn found six cyst-adenomata. These cysts were lined with cuboidal epithelium and show numerous papillary ingrowths simulating, he believes, areas of early growth found in renal hypernephromata.

The main histological argument for the suprarenal origin of renal hypernephroma is the similarity between its histological picture and that of the adrenal cortex; the argument against it is the dissimilarity between its histological picture and that of hypernephroma of the adrenal. Now in the case here presented it seems to me that the carcinomatous areas are very similar indeed to the description and drawings of the four adrenal cases given by Glynn in the *Quarterly Journal of Medicine*.[1] It may be that in the adrenal organ the growth is *usually* carcinomatous whereas in the kidney it is *usually* of an adenomatous type (*i. e.*, resembling normal adrenal cortex), but *may* develop the carcinomatous form and thus present the variegated features of the present case.

THE FORMATION OF ENDOTHELIAL POCKETS IN AORTIC INSUFFICIENCY

ALFRED E. COHN, M.D.

(*From the Hospital of the Rockefeller Institute for Medical Research, New York*)

This heart was taken from a patient who had syphilis. The rhythm of the heart was one of auricular fibrillation. He died of cardiac failure. I should like to call attention to a condition which may be quite common, but has been described only a few times (1–5). It consists in the formation of endothelial pockets which duplicate the shape of the aortic valves; they occur in the long axis of the heart in a line which passes from the aorta downwards and backwards. The individual valves run parallel to each other and open toward the aorta. Their general arrangement is indicated in the photograph of the specimen (figure I). The occurrence of these pockets was first described in 1895 by Zahn (1). Since that time a number of cases have been reported. They have been seen most often in cases of aortic insufficiency. The illustrative figures that have been published resemble this one, but the valve-like formations found here are the least pronounced of those that have been pictured. Their formation is probably due to the fact that the endocardium thickens because the regurgitant stream of blood impinges in an abnormal fashion against the wall of the ventricle, acting as a trauma. The direction of the regurgitant stream depends on which of the aortic cusps is defective. In one case it will be directed toward the left portion of the wall, in another toward the septum. The location of the string of new formed valves varies between these positions and probably depends on direction of the current. The pockets are formed by a continuation of the trauma on the thickening. Similar pockets are found also in the right ventricle as a result of pulmonary regurgitation. When they are formed in the left auricle, as Zahn has reported in one case of mitral insufficiency, the pockets opened toward the apex. They were found once (3) also to open in this direction in the ventricle, but in this case there was no insufficiency, but a very marked aortic stenosis. The opening of the pockets toward the apex is difficult to explain except that they seem to do so toward the blood stream.

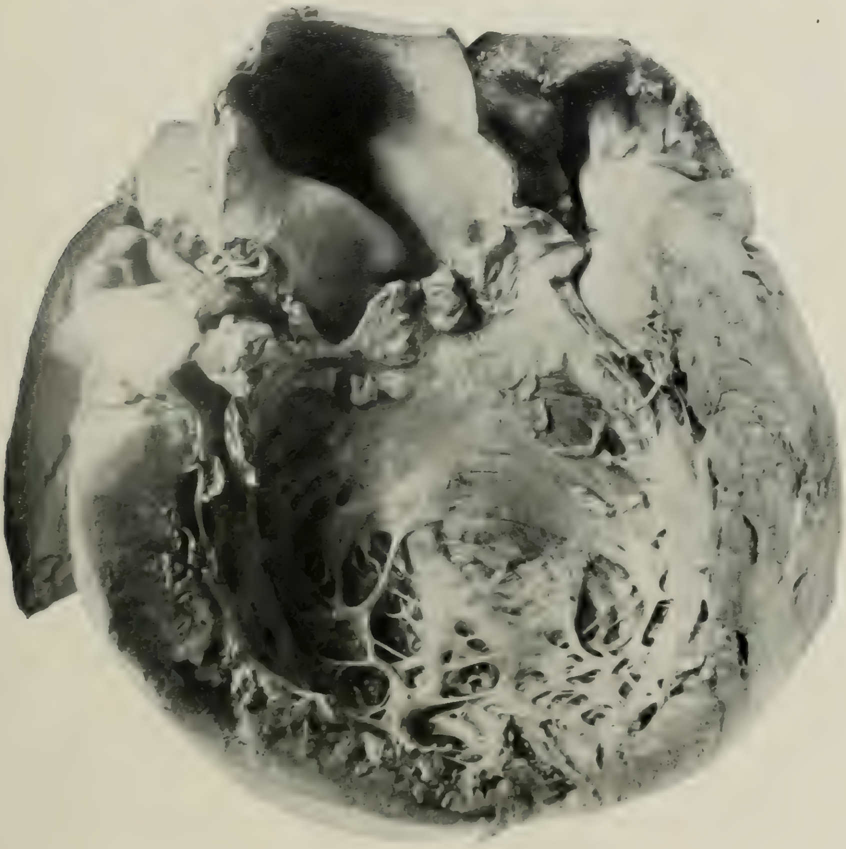

FIG. 1.

Either increased pressure in the left ventricle or some abnormal direction given to the stream may have supplied the trauma.

The cases originally reported by Zahn were not of infectious origin. Whether or not endocardial pockets have relation to an infection of the valve or whether the infection is derived from another focus, either by contact or continuity or metastasis, is difficult to determine. But it is of interest to notice that except in Zahn's cases, the majority occur in aortic insufficiency. These are frequently associated with syphilis, but it is difficult to say whether this may be regarded as the cause.

The formation of endocardial pockets throws an interesting light on the recent discussion relating to the collapsing pulse in aortic insufficiency. Contrary to the traditional view, Dr. H. A. Stewart attempted to show that the collapsing pulse might be due to an escape of the blood to the periphery, not backwards into the left ventricle, but forwards into the dilated arterioles. If the explanation given for the formation of the pockets is correct, a portion of the blood stream, at all events, must regurgitate.

The other lesions present in this heart are a fourth aortic cusp, a large defect in the anterior aortic cusp, probably due to a gumma, and the changes usual in aortic insufficiency.

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Discussion:

Dr. Wadsworth: Was there any evidence of a lesion of syphilis in the endocardium, more particularly in the region where the valves developed, to account possibly for the beginning of the formation of these valves?

Dr. Cohn: There was very marked involvement of the valve. The thickened endocardium shows none of this formation. It is hard to determine whether or not that was syphilis. In some of the other hearts showing endothelial pockets there was no lesion of the valve that could be regarded as syphilitic.

Dr. Libman: I have seen twice a diffuse thickening of the endocardium (both times near the apex) which appeared to be due to syphilis. A case of this sort was reported some years ago in Germany. In Dr. Cohn's case the only lesion that looks as if it might be due to syphilis is the marked shaggy thickening just below the aortic valves. I believe with Dr. Cohn that the valve-like changes which he has found in the endocardium in his case are due to mechanical causes and not due to syphilis.

AN ELECTRIC THERMOREGULATOR

LAWRENCE W. STRONG, M.D.

The ordinary metallic or bimetallic thermoregulators which are on the market for use with electrical heating outfits are rather slow in their action and are apt to spark at the time of making and breaking. This sparking wears out the platinum contact point and may cause it to stick so that the current is not interrupted. The "make" and "break" is not sharp enough. Mercury is more sensitive than metal for a regulator, but there is the same sparking to contend with. To obviate this I had a special mercury thermometer constructed with a platinum point soldered into the glass at 37.5° C. There is also a condenser inserted in such a way that at the instant of the "make" and "break" the current is shunted around in a side circuit from the mercury to the platinum, and this does away with the objectionable spark. This thermometer is connected with one or more dry cells, which furnish a small and easily procured current, better than the use of the main heating current. This current activates a relay below which is an armature held down by a spring. This armature in turn closes the main heating current and here again a condenser is inserted to prevent sparking at this point.

When the heating current is on the armature is down, closing the heating circuit. When the temperature rises to 37.5° the mercury column rises too and completes the circuit in the thermoregulator. This of course raises the armature from the heating circuit and shuts off the power.

I use large glow lamps, but a stove or radiator may be installed. I connect a fan to my heating circuit, so that the temperature is uniformly distributed in the chamber. One may use any closet for an incubator room by putting on ice box doors. This particular thermoregulator was made by Mr. Schramm, of the Claxon Co., Newark.

Discussion:

DR. LARKIN: Can this instrument be used in a large incubating room?

DR. STRONG: Mine is in use in an incubating room. I think it would be adequate for a room as large as this one here, that is if a sufficient number of lamps were used, and an electric fan installed to produce a good circulation of the air.

DR. LARKIN: How many lamps would be necessary for an incubating room of moderate size?

DR. STRONG: Only three are necessary in my room.

DR. LARKIN: What is the temperature of the room?

DR. STRONG: The temperature is 98.6° F. but you can have it made for any temperature.

RELATION OF "TRACHOMA INCLUSIONS" TO CERTAIN BACTERIA GROWING IN INTRACELLULAR NESTS

ANNA WESSELS WILLIAMS

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The following table (table I), arranged in chronological order, gives at a glance the different hypotheses advanced in regard to the nature of "trachoma inclusions." The study of these "inclusions" has brought out an immense bibliography, upon which it is not necessary to dwell in this paper.

Halberstaedter and Prowazek give practically no details of the clinical course of their cases. They simply state that they find the "inclusions" in "acute trachoma." The same criticism may be made of many of the other reports on this subject.

TABLE I

Hypothesis in Regard to Nature of "Trachoma Inclusions"

I. Peculiar organisms called chlamydozoa, causing "trachoma."	Halberstaedter and Prowazek, 1907.
II. Involuted gonococci "microgonococci," causing "trachoma."	Herzog, 1910.
III. Cell Degenerations.	Addario, 1910.
IV. An unknown microorganism having nothing to do with trachoma, causing conjunctivitis.	Noguchi and Cohen, 1910.
V. Certain bacteria growing in intracellular nests—hemoglobinophilic bacilli in certain cases of papillary conjunctivitis ("acute trachoma"), gonococci in gonorrhœal conjunctivitis, and probably one or two other species of bacteria in certain other cases.	Williams, 1912.
VI. A specific bacterium causing "inclusion conjunctivitis."	Noguchi and Cohen, 1913.

We have found in our study of trachoma and allied conditions in New York City public school children[1] that cases of conjunctivitis called by ophthalmologists "acute trachoma" and showing "trachoma inclusions" mean clinically the presence of follicles and papillary thickening in the conjunctiva, accompanied by more or less secretion. The vast majority of these cases in our experience, after a subacute or chronic course averaging nine months, present apparently normal conjunctivas.

So far then our findings agree with those of Noguchi and Cohen. That is, many cases of "inclusion conjunctiva," as they have been called, run a comparatively benign course to cure without the formation of cicatricial tissue. Therefore these cases apparently have nothing to do with "classic trachoma."

In our studies we demonstrated further the interesting fact that hemoglobinophilic bacilli were found coincidently with trachoma inclusions in these cases of papillary conjunctivitis as we began to call them, when we found that practically 100 per cent. of cases examined from or near the beginning of the disease were getting well without scar tissue. As the cases began to recover both bacilli and inclusions were demonstrated irregularly and as some of our cases were not examined until they were beginning to recover this massed table (table II) does not show as high a percentage of incidence as it might.

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[1] To appear in the March, 1914, number of the *Journal of Infectious Diseases.*

TABLE II

Coincidence of Bacteria and Inclusions

Clinical Diagnosis	No. of Cases	Hemoglobinophilic Bacilli		"Trachoma Inclusions"	Gonococci		Cured Without Cicatricial Tissue
		Typical	Transition Forms		Typical	Transition Forms	
Papillary conjunctivitis....	237	177	134	184	0	0	90 per cent. in 9 mos. Average.
Acute catarrhal conjunctivitis..................	50	44	35	8	0	0	100 per cent. in 2 mos. Average.
Ophthalmia neonatorum...	38	3	1	9	19	13	95 per cent. in 2 mos. Average.

In studying closely the morphology in cultures of our hemoglobinophilic bacilli, we were struck by the fact that they frequently grow in more or less dense clumps of extremely minute coccoid forms. This led us to the conclusion that possibly they formed the "trachoma or Prowazek inclusions" and when we found that these bacilli and the inclusions were found coincidently and repeatedly in so many cases diagnosed as acute papillary trachoma the possibility became a probability. So we proceeded to study the morphology of the cultures more minutely. Each culture was kept under observation for many days. Each day spreads were made from the same culture and stained by Giemsa's method exactly as we stain our direct films for the detection of the "trachoma inclusions." In examining these smears we found cultures of these bacilli, as they grow older, alter significantly both in their morphology and in their staining reactions to Giemsa. Twenty-four hour cultures show dense clumps of short bacilloid or coccoid forms taking a comparatively deep blue stain exactly as do the early granules of the "trachoma inclusions" (the "initial bodies of Lindner"). The morphology of course varies somewhat with the age of the inoculated culture, the date from isolation, the kind of medium, and the strain. In

48 hours the forms become somewhat more irregular. Then in a very few days most of the bacilli have become extremely minute, many showing only as reddish granules (the "elementary bodies of Prowazek"), while scattered through the culture are swollen spheroidal bodies taking a fainter clear blue stain (the larger "initial bodies of *Prowazck"*), in some of which are minute reddish granules (resembling the "elementary bodies of Prowazek"). A number of irregular light blue bodies may be also scattered through the culture. Where the bacteria are densely grouped more red granules may appear in the center of the group than at the periphery and more blue bodies at the periphery than in the center. In short, practically all of the changes described by Prowazek and others as characteristic of "trachoma inclusions" are seen in the growing cultures of these hemoglobinophilic bacilli (Demonstration chart).

Similar day-to-day studies were undertaken with the other types of bacteria found most frequently in the eyes diagnosed as trachoma, *e. g.*, with streptococci, staphylococci, gonococci, xerosis bacilli, micrococcus tetragenus and a minute Gram-negative non-hemoglobinophilic bacillus not before described, found in a few cases of papillary conjunctivitis, but in none of these varieties except the gonococcus were similar changes found in the same marked degree. Cultures of the tetragenus coccus, isolated repeatedly from a case of chronic conjunctivitis, showed some dense bunches but practically no red granules. This corresponded with what was found in direct smear from the case. Many of the epithelial cells contained the tetrads, some distinct and some in dense clumps, approaching in morphology the "early trachoma inclusions" found in ophthalmia neonatorum, but no forms containing reddish granules were seen.

The fact that the gonococcus cultures showed so definitely appearances similar to the trachoma inclusions was most interesting considering the claims made by so many that trachoma inclusions are found in cases of ophthalmia neonatorum, and that the gonococcus is the cause of trachoma. It led us to make a special study of a series of ophthalmia neonatorum cases, thirty-eight in all. The cultural technic employed was the same as that used for the other cases. In most of these thirty-eight cases, treatment had already been begun when our cultures and films were made; in fact, many of the cases were already near recovery, so we were not able to get a complete cultural and morphologic picture; however, the results are suggestive. Gonococci were demonstrated by us in both smears and cultures in ten cases, in smears alone in nine cases, in cultures alone in three cases, and in neither culture nor smear in sixteen cases. Most of the cases giving negative results and some of the others were examined several times. This picture of irregular results is one that might be expected from recovering cases. From only three of these cases were hemoglobinophilic bacilli isolated. In nine cases definite "trachoma inclusions" were seen, in only one of which hemoglobinophilic bacilli were found as well, while in seven, gonococci were found. In six others showing transitional forms of inclusions, gonococci were also found. In two showing "trachoma inclusions" no definite organisms were demonstrated in either smear or culture.

A minute study of the direct smears from these eyes apparently throws more light on the subject. In all of the inclusion cases where gonococci are found, apparent transition forms between gonococcus and inclusion are very evident, and we find from a further microscopic study of these slides that the inclusions on the whole present certain characteristics different from those found in our series from school children, produced, according to our hypothesis, by nests of growing hemoglobinophilic bacilli. The inclusions produced, as we think, by the gonococci are characterized in *early* stages by being more definitely coccus-like at the periphery of the clump and more densely clumped in the center, and in *later* stages by showing on the whole smaller red granules than those produced by the hemoglobinophilic bacilli. These same differences are observed in a comparative day-to-day study of pure cultures of these two species of bacteria. We have found different strains of each species vary in size of granules produced, so the second characteristic is not a clear cut one.

From this comparative morphologic study of inclusions and cultures as well as from the clinical studies we have reached the following conclusions:

1. The well-known power of the epithelial cells of the conjunctiva as well as epithelial cells of other parts of the body to harbor foreign invaders (*e. g.*, erythrocytes, leucocytes, several species of microorganisms, notably gonococci, and hemoglobinophilic bacilli) may be in evidence in any stage of inflammation.

2. Under certain conditions of infection, chief of which are, probably, a lessened virulence on the part of the invading organism and a lessened susceptibility on the part of the invaded cells, or one or the other of these conditions, some invaders, notably gonococci and hemoglobinophilic bacilli, may grow to a varying extent within the cells in groups or nests and thus produce the characteristic changes described as "trachoma inclusions."

3. Under these conditions, too, growth of the invader may not be limited to the epithelium alone but may extend to the deeper tissues. According to the extent and kind of irritation produced by such growth, a variable degree of proliferating inflammation of the surrounding tissue may be established, which later, if unchecked, may go on to the formation of fibrous tissue.

If it be true that both gonococci and hemoglobinophilic bacilli (and possibly other species of bacteria) may grow in epithelial cells in nests presenting a morphologic cycle similar to that described for the "trachoma inclusions"; it explains, as I have pointed out, the contradictory findings reported in cases of "inclusion ophthalmia," gonorrhœal conjunctivitis and "inclusion inflammation" in the mucous membranes of urethra and vagina ("trachoma of the urethra or vagina" (?)) and of other parts of the body. That is, in a large number of cases of "papillary conjunctivitis" and a certain number of cases of ophthalmia neonatorum, as well as in a certain number of cases of inflammation of the mucous membranes of other parts of the body (*e. g.*, vagina, urethra), the "trachoma inclusions" found are due to one or more varieties of hemoglobinophilic bacilli; in a certain number of cases of gonorrhœal ophthalmia as well as in gonorrhœal inflammation of the mucous membranes of other parts of body, the "trachoma inclusions" are due to the gonococcus.

According to one of the later reports of Prowazek and the recent report of Noguchi, certain "inclusion conjunctivitis" cases may be caused by other microorganisms than the two mentioned above.

Discussion:

DR. WADSWORTH: These forms certainly resemble one another morphologically very closely. Has Dr. Williams tested many different methods of staining to determine whether or not there was any difference in the staining reactions?

DR. WILLIAMS: We have used several stains in this comparative study, and with each cultures and "inclusions" stained similarly. With Gram cultures and "inclusions" are negative. Giemsa's stain was found to be the most distinctive.

ON THE SO-CALLED HEMO-URO-CHROME TEST FOR CANCER

E. E. BUTTERFIELD, M.D.

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In the *American Journal of the Medical Sciences*, June, 1913, there appeared an article by T. G. Davis entitled, "Hemo-urochrome: a New Laboratory Test for Cancer and Sarcoma, etc." The test consists in heating together 100 c.c. of urine and 10 c.c. of hydrochloric acid to the boiling point and then shaking the cooled mixture with ether. Davis states that the ether is occasionally colored pink or even red and that "by far the greatest amount and intensity of color obtained by the process described was found in the urine of patients suffering from malignant disease, cancer and sarcoma." The paper is expressed in such a vague and confused manner that we should have taken little notice of it had not our attention been called to the fact that on one of the medical divisions of Bellevue Hospital the test was being used for diagnostic purposes by one of the attending physicians. Furthermore in several instances operation was advised on the strength of a positive outcome of this test. We then decided to look more closely into the matter.

First the test was tried on an undoubted case of carcinoma of the stomach in which the diagnosis was subsequently confirmed at autopsy; result of test, positive. A case of suspected carcinoma in which operation had been advised on the strength of a positive result with this test was then examined and the positive result confirmed. The similarity of the test with the older methods of preparing indigo red from urines rich in indican suggested at once the possibility of the red color of the ether extract being due to indigo red. Tested for indican both of the previously mentioned urines gave an intense positive reaction with Obermayer's reagent. A normal urine was then tested by the Davis method and a positive reaction was obtained which surpassed that of either of the other cases in intensity. The supposedly normal urine had been as a rule free of indican. But on testing for indican in a sample of the portion collected for the Davis test an intense indican reaction was obtained with the Obermayer reagent.

In order to establish more firmly the concurrence of indicanuria and the red color of the ether extract after boiling the urine with hydrochloric acid, a number of other urines giving a positive Obermayer reaction were also tested by the Davis method. In every case in which indigo blue was demonstrated by the Obermayer method, the Davis test was also positive.

Larger quantities of the three urines first examined were then treated with hydrochloric acid and heat, extracted with ether in a separatory funnel, and the ether extract evaporated to dryness. In each case a cherry red residue was obtained which corresponded in its solubilities to indigo red. The opinion was then expressed that the red color of the ether extract in such cases was due to indigo red. In spite of this the second case, *i. e.*, the case of suspected carcinoma was operated on. Nothing was found. The patient died. At autopsy no carcinoma was to be found; there was a general peritonitis. This was conclusive evidence that the test had nothing to do with carcinoma *per se*.

The urine from the first case of carcinoma of the stomach was then collected for several weeks and an attempt made to isolate the red substance in a crystalline state. The so-called "hemo-uro-chrome" test was always positive, likewise the Obermayer reaction. The method of isolation was essentially that of Rosin,[1] inasmuch as the substitution of HNO_3 for HCl gives a larger yield of the red substance. In either case the red substance shows the same spectrum and possesses the properties of solubility. From this urine of the case of carcinoma of the stomach it was possible to isolate by the procedure of Rosin a few milligrams of dried substance with the following properties:

1. On heating with glucose in alkaline solution the red alcoholic solution turns colorless or faintly yellow. On shaking with air the solution again becomes pink or purple. The color play can be repeated at will.
2. The substance sublimes at 300° C. into a cherry red vapor.
3. The absorption spectrum of the ethereal solution shows a broad band with poorly defined margins extending from the region of the Na-line over to violet. In addition there is a narrow line between 610 and 620 $\mu\mu$ which indicates the presence of a small amount of indigo blue.
4. The substance is insoluble in water; soluble in chloroform, ether, anilin, ethyl alcohol, amyl alcohol, and glacial acetic acid.

CONCLUSION

The red color of the ether extract after boiling urine with hydrochloric acid in cases of carcinoma is caused by the presence of indigo red. The same reaction can be obtained with any urine containing a sufficient quantity of indican. The so-called hemo-uro-chrome test is therefore simply a reaction for urinary indican and stands in no direct relation to the presence of cancer.

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[1] Rosin, *Virchow's Archiv*, CXXIII, p. 539.

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Dr. A. M. Pappenheimer, *President*

APPARATUS AND TECHNIQUE FOR TAKING BLOOD

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(Department of Laboratories, New York Post-Graduate Medical School and Hospital)

I

Blood culture has now become such a common procedure that any methods which may simplify the technique seem distinctly worth while. With this object in view we have tried a variety of apparatus and have finally hit upon one which has proven fairly satisfactory.

In spite of various modifications and substitutes, syringes are probably most commonly used for this purpose and consist largely of three types, viz., the all glass (Luer pattern), the glass with metal plunger (Reichert), and the glass with asbestos-packed plunger (Behring antitoxin), all of which have individual faults as well as certain general disadvantages.

The first of these has seemed to us to be the most desirable as it can be most easily cleaned and sterilized, but it has the serious objection of being extremely fragile, for, whether it is boiled in an open container or steamed under pressure, sooner or later it either cracks outright or chips about the needle attachment so that the connection is made imperfect, or it cracks and eventually shatters on the ground surfaces, thus preventing the plunger from sliding smoothly. It was our custom to autoclave the plunger and barrel separately in glass containers so that they might be kept ready for use at any time. The glass with metal plunger is not well adapted to being kept in a sterile condition as the moisture which necessarily collects during autoclaving will soon attack the metal. On the other hand, if boiled just previous to use it requires a considerable length of time to cool and the plunger will not fit the barrel properly until it is cool. It also has a tendency to "stick" especially when the blood is on the verge of clotting. Syringes with asbestos-packed plungers are difficult to clean and sterilize and as they will not work when dry, cannot be kept permanently sterile.

But whatever syringe is selected it is necessary to carry the media to be inoculated to the bedside or transfer the blood immediately to a test tube containing some solution to prevent fibrin formation, either of which procedures is rather cumbersome, to say nothing of the chances of contamination which frequently exist under such circumstances. These objections have been pointed out so that certain advantages of the apparatus here described may be more apparent.

The apparatus consists simply of a test tube (fig. 1), 20 mm. in diameter and 150 mm. in length with a piece of capillary tubing about 70 mm. long welded to it at right angles at a point slightly below the juncture of the middle and upper thirds and with the free end of the tubing ground to fit the standard Luer needle. Previous to sterilization, the tube is plugged with cotton in the usual way and a small test tube placed over the arm and held in position by fitting it snugly over cotton wound about the capillary near its juncture with the large test tube (fig. 1). In this way the entire arm and the inside of the test tube are well protected and may be kept indefinitely without danger of contamination. The needles, after being thoroughly cleaned, dried and sharpened, are put in small tubes containing about 1 c.c. of liquid paraffin and a little glass wool at the bottom to protect the point.

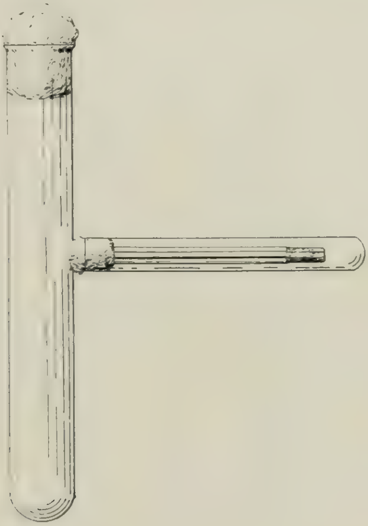

FIG. 1.

These are all sterilized in the dry oven. By this method they are kept in excellent condition and the whole apparatus is ready for use at any moment.

When a blood culture is to be taken, 1 c.c. of sterile 10 per cent. sodium citrate solution is put in the tube, the small tube protecting the arm removed, and the needle attached by inserting the end of the ground capillary into the needle socket. To assist the flow of blood by suction a one-hole rubber stopper (fig. 2) with glass and rubber tubing attachment may be shoved in on top of the cotton plug. After the blood is withdrawn it is shaken slightly to mix it with the citrate and taken to the laboratory and cultured.

The same result may be accomplished by adding .05 to .1 gm. of powdered potassium oxalate to each tube and sterilizing it at the same time in the oven. In this case care should be taken to shake the blood well so as to insure solution and thorough mixture of the oxalate. Powdered citrate, because of the large amount of water of crystallization, is not so satisfactory for this purpose as it forms a hard mass on being heated and goes into solution with difficulty.

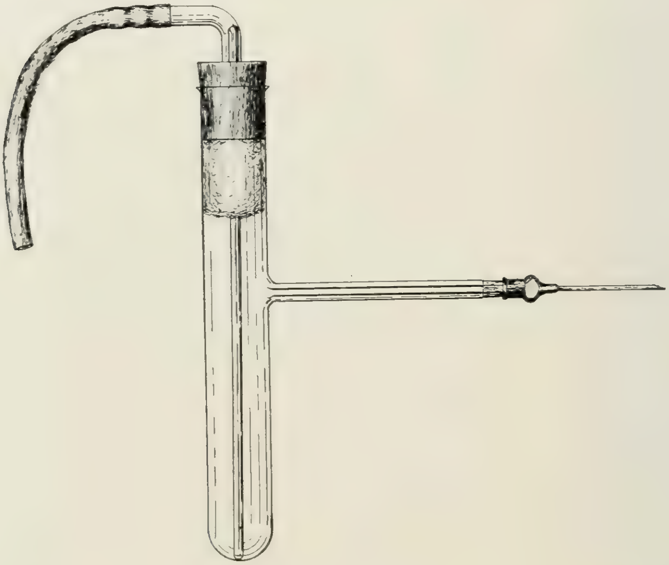

FIG. 2.

If defibrination is desired it may be easily accomplished by a glass rod which can be wound within the cotton plug and allowed to extend 2–3 cm. above the mouth of the tube (fig. 2). The glass tubing in the rubber stopper should be of sufficient caliber to let the rod pass within it.

The apparatus has proven so simple and convenient that the laboratory internes employ it exclusively for taking blood for all purposes, Wassermann reactions included, in which case the blood is of course allowed to clot and the serum withdrawn later. We have had them made at a cost of thirty cents each.

The principal advantages of the apparatus may be summarized as follows:

(1) It is easily cleaned and may be sterilized by dry heat and kept ready for use for an indefinite period.

(2) The blood may be citrated, defibrinated or allowed to clot in the same container and without exposure.

(3) Blood for culture may be conveniently transported to the laboratory without danger of contamination.

(4) It is durable and cheap.

II

Modifications of this apparatus have supplanted the syringe in many other of our laboratory procedures. For example, we had been accustomed to take complement with a syringe by means of puncture of the guinea-pig's heart, but we now use a modification (fig. 3) of the apparatus already described. The modification simply consists in the needle connection being welded to the bottom of the test tube instead of to the side. The same method of producing suction is used as in the blood-culture tube. After the desired quantity of blood is sucked up it is immediately blown out into another container to allow the serum to separate. This same device may be employed in securing anti-sera in which the sterility of the serum is necessary.

In order to eliminate the danger of contamination incident to the transfer of the blood to another container, the following slight modification (fig. 3) has been found convenient. The needle instead of being attached directly to the tube is joined to it by means of a rubber connection. This is done so that when the desired amount of blood is drawn the rubber may be clamped. In this way subsequent defibrination may be performed or the serum allowed to separate by clotting in the original receptacle.

III

It is our custom to handle blood and other sterile fluids in the Pasteur bulb-pipettes. Formerly the usual method was to bleed the animal from the carotid by inserting a glass capillary drawn from what may be described as a very large test tube, that is, sufficiently large to hold the maximum yield of the animal. After defibrination, the blood was sucked into the well known bulb-pipette and was thus kept in a form insuring its sterility and very convenient for use from time to time as the occasion demanded.

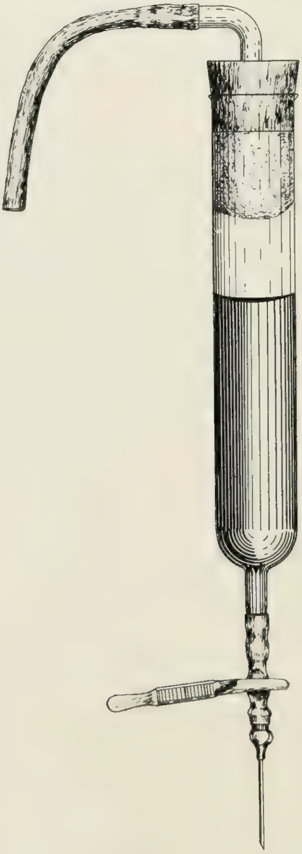

FIG. 3.

This method, of course, sacrifices the animal and in laboratories where a constant fresh supply of blood is necessary, the sacrifice of an unavoidably large number of animals involves a considerable expense and at times trouble in securing them. To avoid this item and disagreeable feature we have for some time used the heart puncture with excellent results in every respect. Accordingly, we have combined the heart puncture with the excellent Pasteur bulb-pipette in the following way:

The glass tubing is drawn out in the ordinary way but is not sealed in the middle as is the usual method to secure two pipettes, but at one end so that a single pipette of double capillary length is the result. Further, the sealing is done at such a point as to give an enlargement at the end of the capillary sufficient to fit tightly within the rubber attachment of the needle. The bulb is now blown and the capillary is bent first near the bulb at a right angle and again at its middle at an angle as shown in the illustration (fig. 4). The sealed tip is scratched with a file and broken off; this open end is sterilized in the flame and the previously sterilized rubber and needle is attached. Then 5 c.c. of sterile sodium citrate is drawn through the needle into the bulb, heart puncture is done and about 50 c.c. of blood drawn in and mixed with the citrate in the bulb. The capillary is then sealed at the point where it is bent upon itself as indicated by the arrow in the illustration, thus giving finally the ordinary bulb-pipette with blood ready for use.

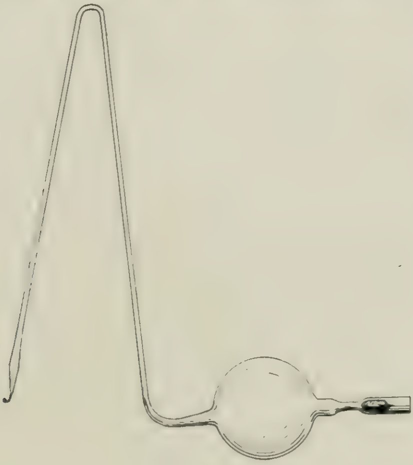

FIG. 4.

Discussion:

Dr. Pappenheimer: The only possible disadvantage which occurs to me is the possibility of breaking the arm at the place where it is fused to the main stem. Does not that sometimes happen when forcing the needle through a thick skin?

Dr. Taylor: At first when we made the apparatus it did break on sterilization about the joint but this was due to not subsequently putting them in an annealing oven; but if this is done they stand sterilization perfectly. When inserting the needle the capillary arm is held and the force applied there instead of upon the test tube so that no strain is put upon the joint. We have never had any such experience as breaking the tube on account of a difficult skin.

GIANT-CELL TUMOR OF FEMUR

DR. O. S. HILLMAN

(*Pathological Laboratory, Post-Graduate Medical School and Hospital*)

Giant-cell tumors, speaking generally, are fairly common, occurring in the form of both sarcomata and epithelial new growths. The origin of the giant cells and their mode of formation is a question of interest in such tumors, as our conception of their histopathology is influenced to a certain extent by the significance which we attach to these large cells.

There are a few well-defined varieties of sarcoma in which the giant cells are due to the rapid division of the tumor cells composing the growth, with the resultant formation of multiple mitoses and multilobulated nuclei. Giant cells of this type are not infrequently seen in rapidly growing fibrosarcomata, particularly those arising from the periosteum and aponeuroses. These large multinucleated cells are not necessarily a prominent feature of this kind of tumor, and do not constitute the type usually spoken of as giant cell sarcoma. Giant cells originating in the way above mentioned may be regarded as true tumor giant cells, and are to be distinguished from so-called foreign body giant cells which are formed under a great variety of conditions, particularly in reparative processes of bone.

The term giant-cell sarcoma (for it is in sarcoma that the usage of this descriptive phrase finds its greatest vogue) is applied to those lesions arising from mesoblastic tissue in various parts of the body, but especially from bone. In the extremities of the long bones there occurs a type of growth which presumably develops in the marrow or cancellous tissue, and which results in the formation of a fusiform swelling, with disintegration and resorption of the trabecular and cortical bony framework. This lesion is frequently spoken of as a medullary or myelogenous giant cell sarcoma. The specimen shown here is, I believe, one in which we are accustomed to make this microscopic diagnosis.

The *clinical history* of the case is briefly as follows: Male, white, age 63 years. Family history negative. Four years previous to operation (which was performed in October, 1913), he injured his right knee; he felt immediate pain and noticed a swelling. He was confined to bed for two weeks, during which time the swelling was aspirated and a tablespoonful of tarry fluid removed. Two months following the injury an X-ray picture was taken and showed patchy rarefaction and irregularity in contour of the lower extremity of the femur. Three months after injury he resumed his occupation as a packer and shipper of china. He remained well until two years prior to the operation, when he fell a distance of 12 feet from the top of a lumber pile, injuring the same knee, and limped thereafter. In December, 1912, he was compelled to give up work on account of disability and pain—standing or walking. In May, 1913, the knee was aspirated and a small amount of blood was removed. From this time on motion in the joint was absent.

Physical examination in August, 1913, revealed the following condition: Lower third of right thigh and knee-joint enlarged.

MEASUREMENTS.

	Inches.
Right knee	17
Left knee	13½
Right thigh (middle)	16½
Left thigh (middle)	15

Veins dilated over affected area; slight redness and heat. Wassermann reaction was negative. General arteriosclerosis present.

The X-ray diagnosis at this time was central sarcoma of lower end of femur.

The patient was operated upon and a vertical incision six inches long was made over the anterior surface of the lower third of the thigh. The lesion came into view immediately upon cutting through the edematous soft parts. The anterior cortical bony wall of the lower extremity of the femur was destroyed

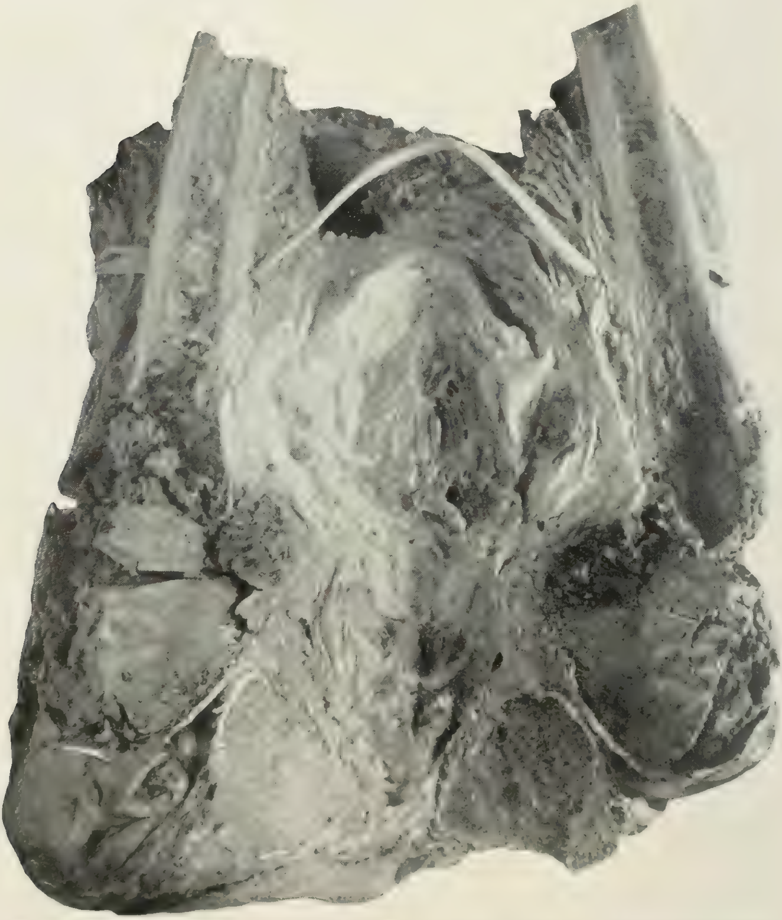

FIG. 1. Lower end of femur split open longitudinally, showing position of lesion.

over an area the size of a goose egg. The mass presenting was very hemorrhagic, reddish-brown in color, quite friable, but firm in consistency; the anterior surface was smooth, and was even with, but did not bulge through the opening in the bone.

Owing to the patient's enfeebled condition and the evident poor vitality of the edematous soft parts surrounding the lesion, amputation of the thigh through its upper third was performed.

Examination of the gross specimen which was brought to the laboratory in a comparatively fresh condition revealed the following appearance: The lesion is located in the lower end of the femur and for the most part in the condyles. It measures approximately 8 × 7 cm. in the vertical and transverse diameter, respectively. On splitting the specimen open longitudinally (fig. 1), it is seen that the lesion is rather sharply circumscribed. It is limited below only by the articular cartilages of the condyles; posteriorly it abuts against the fatty tissue of the popliteal space, the posterior surface of the femur being eroded in this region over a distance of about 7 cm. Above, the lesion is fairly well marked off from the medulla of the shaft. On the outside, the bone is eroded and the tumor mass is limited by the stretched-out muscle layers, and also by a connective tissue membrane continuous with the muscle sheaths. On the inside it is limited by muscle and by the thin bony wall of the excavated internal condyle. There is no extension of the process into the knee-joint. The cut surface of the growth shows a color varying from a reddish-black (areas of hemorrhage) to a brownish-gray and a pale yellow. It looks very much like organizing blood clot. The bone marrow above the lesion is yellow in color, soft and edematous. Fragments of the main mass when shelled out of the bony cavity are firm but friable. These separated pieces contain many small softened areas. About the periphery of the mass, thin layers and spicules of soft yellowish-red bone are seen in contact with the bony cavity, which is rough, eroded and deeply hemorrhagic.

Microscopic examination shows that the bulk of the growth is made up of highly vascular tissue resembling granulation tissue. Throughout are found many multinucleated giant cells which are located for the most part in lacunæ some of which are formed apparently from dilated blood spaces. Giant cells are seen containing as many as 40–50 nuclei, while others contain only 2 or 3. Most of the giant cells show an abundant cytoplasm which takes the acid stains deeply, and which shows a reticulated or vacuolated appearance in several of the cells. Small fat globules are detected in and about the cytoplasm of many of the giant cells; fat is also scattered elsewhere throughout the growth. The intervening supporting stroma is made up largely of fibroblasts with a small sprinkling of polygonal cells. Between the fibroblasts are seen many collagen fibrils demonstrable with aniline blue connective tissue stain. Sections of the better preserved portions stained with phosphotungstic acid hematoxylin show fibroglia fibrils coming off from the fibroblasts. Mitotic figures are very rare in the stroma cells. A great deal of the growth is necrotic, particularly about the hemorrhagic areas. Bone is not found in the central portion of the mass, but about the periphery where the mass comes in contact with the bony shell evidences of disintegration of the osseous tissue are seen, together with small foci of hemorrhage, proliferation of fibroblasts and endothelial cells, and lymphocytic infiltration, particularly in the medullary spaces.

The giant cells conform to the characteristics of foreign body giant cells found so frequently in bone lesions of this type, and are in all probability formed, as Mallory has suggested, from endothelial leucocytes which have become fused together about some material, such as lime salts or the products of cell distintegration. If viewed in this light they may be considered as scavengers whose function it is to remove the foreign substance about which they form.

The microscopic picture strongly suggests a reparative process in response to some injurious agent.

REMARKS

It has been maintained for some time that trauma plays an important part in starting bone lesions of this type. Injury to the fine trabecular structure of a bone may cause rupture of the delicate blood sinuses resulting in hemorrhage and pressure necrosis. The hemorrhage and necrotic tissue cells attract endothelial cells as phagocytes, which fuse to form the giant cells; the fibrin present probably stimulates a fibroblastic proliferation. The process, once started in this way, goes on, and in the course of its development, the bony structure is disintegrated and replaced by this primitive type of granulation-like tissue. It is not proven that such is the actual manner in which the pathologic changes have occurred in this or similar cases, but a consideration of the histology and histopathology involved, suggests that the lesion could be brought about in this way.

If such a conception is tenable, it would suggest that here we are not necessarily dealing with a true neoplasm, but rather with a low grade inflammatory reaction (in the strict pathologic sense of the term) to some injury.

Inasmuch as the growth shows a tendency to continuous erosion and resorption of the bony framework, with a steady increase in size, and also particularly on account of the widespread giant cell formation, it is generally classified as a sarcoma.

The importance of diagnosis in lesions of this kind is evident, as the treatment will vary according to our conception of the pathologic process.

(Lantern slides were shown, illustrating the important pathological and clinical features of the case.)

Discussion:

Dr. Lambert: I was interested in what Dr. Hillman had to say about the two types of giant cells found in tumors, the true tumor giant cell and the foreign body giant cell. I can hardly agree with his statement that the presence of mitoses is one of the distinguishing features of the former type. It has been my experience to observe very rarely mitotic figures in giant cells of either type.

I would also like to express my disagreement with the idea of the stimulating action of fibrin on the growth of connective tissue. Certainly in the ordinary pneumonic consolidation of the lung we have an instance where there is an enormous outpouring of fibrin which is only exceptionally followed by connective tissue overgrowth.

Dr. Pappenheimer: In regard to Dr. Hillman's interpretation of the giant cells in this tumor as foreign body giant cells, I wish to ask what relation he believes them to bear to the multinuclear osteoclasts, and also what direct histological evidence there is that they are foreign body giant cells; whether they contain calcareous or cellular inclusions, and whether they arrange themselves about inert particles after the fashion of foreign body giant cells elsewhere?

Dr. Hillman: It is oftentimes difficult to demonstrate the differential points which are maintained for the distinction between these two types of giant cells. This depends to a certain extent upon the character of the tissue —whether it is freshly fixed or otherwise. I have seen true tumor giant cells to best advantage in a rapidly growing fibrosarcoma, and if a tumor of this kind should be growing in or about bone, foreign body giant cells are also to be found occasionally. In the tumor giant cells, the nuclei are not generally as numerous and the cytoplasm is not as deeply stained and abundant as is the case in the typical foreign body giant cell. The evidence of nuclear mitoses is, as I have observed in some of Dr. Mallory's sections, an important distinguishing feature. As Dr. Lambert has mentioned, it is quite true that we do not see multiple mitoses in typical large giant cells so very often; the other type of cell is more common.

In regard to the relationship between osteoclasts and giant cells, some pathologists are of the opinion that the osteoclasts are foreign body giant cells formed by the fusion of endothelial leucocytes for the purpose of bone disintegration.

In many foreign body giant cells there can be demonstrated substances of various kinds, such as cholesterin and fat crystals, lime salts, products of cell disintegration, etc. In the specimen which I have presented, the principal attraction to endothelial cells seems to be fat, as the giant cells are seen to contain fat droplets in considerable number within the cytoplasm of the cells. This is especially noticed where the giant cells are the most numerous, viz., about the necrotic areas, which are filled with fat, as demonstrated with Scharlach, R., in frozen sections.

In connection with fibrin as a stimulant to connective tissue proliferation, what I had in mind was not so much the abundant fibrin production as seen in lobar pneumonia which undergoes resolution when the patient recovers, but rather those cases in which resolution is delayed, and where the fibrin is not completely digested by the leucocytes. In such lungs, organization of the fibrin may be seen. In the sections of this giant cell tumor, which have been stained with aniline blue connective tissue stain and with phosphotungstic acid, collagen and fibroglia fibrils can be detected throughout the areas of fibroblastic proliferation, in the immediate neighborhood of the fibrin.

HEMORRHAGIC INFLAMMATION OF THE SMALL INTESTINE WITH ULCERATION AND PERITONITIS

A. R. LAMB, M.D.[1]

(*From the Department of Pathology of the Presbyterian Hospital*)

The case was that of a woman patient, 38 years old. She had previously been healthy. Two days before admission to the hospital she developed marked abdominal pain, without rigidity. An exploratory laparotomy was performed and an early peritonitis was found without any apparent causative factor. The appendix was removed; it proved to be normal. The patient went into shock after operation and died a few hours later. Smears from the peritoneal exudate showed a few Gram positive diplococci, which were culturally identified as pneumococci. The lungs were extremely oedematous with a few areas of consolidation. A diphtheroid bacillus in considerable numbers was found in the lungs and also in sections taken from the intestine. This organism was isolated but lost in the subsequent transplantation. The pneumococcus was recovered and identified. Throughout the greater portion of the ileum there were numerous hemorrhages in the mucous membrane. They appeared almost like thrombi and some of them have begun to show superficial ulceration. On the serous surface there was considerable ecchymosis. The specimens have begun to lose their brilliancy of color, but when they were first removed the hemorrhagic areas were bright red. One finds very fresh localized hemorrhages for the most part, without inflammatory reaction and situated within the mucosa. In some places they have torn through the muscularis mucosa and in some places, also, there is a loss of the overlying epithelium and an early inflammatory reaction. Throughout the epithellium a large number of diphtheroid bacilli are found but these are not especially abundant in the region of the hemorrhages.

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[1] This communication was presented for Dr. Lamb by Dr. A. M. Pappenheimer.

This case therefore presents an unusual type of intestinal inflammation of obscure etiology. Dr. Lamb does not feel that the diphtheroid bacilli were necessarily essential to the production of the lesion; they may have been secondary invaders.

Discussion:

Dr. Norris: Where did the pneumococci come from?

Dr. Pappenheimer: They came from the peritoneal exudate.

Dr. Norris: It is curious how you do get capillary thrombi in cases of infection. It is not clear why you should get capillary hemorrhages or petechial eruptions or emboli in the submucosa. I saw a case four months ago at Bellevue. A staphylococcus was found. The hemorrhages were quite similar to this one and were found in large numbers. Why they should in some cases involve the intestinal mucosa is not known.

Dr. Pappenheimer: The lesions in this case are not true thrombi, but simple hemorrhages.

CONGENITAL VARIATIONS IN THE PERITONEAL RELATIONS OF THE ASCENDING COLON, CÆCUM AND TERMINAL ILEUM

S. C. HARVEY, M.D.

(*From the Department of Pathology of the College of Physicians and Surgeons*)

In the last five years increasing attention has been directed to certain signs and symptoms in the right side of the abdomen which have been usually interpreted as indicative of chronic appendicitis. Such conditions are the "cæcum mobile" of Wilms (1), the ileal band of Lane (2) and the "membranous pericolitis" of Jackson (3). The data as regards frequency of occurrence of these conditions has been in great part derived from instances seen at operation. Likewise the etiology of the adhesions about the colon and ileum has been dependent upon this source of information and in consequence there has arisen two theories as to their origin. The one, as represented by Pilcher (4), Gerster (5) and others, regards them as formed by a previous subacute inflammation, the other, as supported by C. H. Mayo (6), Flint (7), Reid (8) and others, asserts them to be but developmental defects. The examination of fœtuses, notably by Flint, has given greater weight to the later hypothesis.

In order to obtain a larger amount of data bearing upon the frequency of occurrence of these abnormalities and their mode of origin, 105 children varying in age from birth to three years and seven months were examined post mortem at the New York Foundling Hospital. Of these 43 were in the first two months of life, 28 between three and seven months of age and 24, seven months old or over.

The conditions looked for were: abnormal mobility and position of the cæcum, undue development of the cysto-colic fold, abnormal omental attachments, pericolic membranes, pericæcal membranes, appendicular membranes, and Lane's band. The frequency of occurrence was as follows:

	Number Found.	Per Cent.
Cæcal displacement	32	30
Cysto-colic fold	20	19
Omental attachments	10	9
Pericolic membranes	17	16
Pericæcal membranes	13	12
Appendicular membranes	8	8
Lane's bands	9	9

Of these abnormalities 15, or 14 per cent., were sufficient to cause some angulation or kinking of the colon, appendix or ileum. The variations in the position of the cæcum as well as of the cysto-colic fold were of the same frequency in those under three months as in those over this age. The membranous structures, however, seemed rather to decrease with the age, and this was especially true of Lane's band. As regards sex, abnormalities in the position of the cæcum were more frequent in the female and this was also true of Lane's band. On the other hand, the remaining membranes were slightly more prevalent in the male.

From a consideration of the above data one must conclude that these peritoneal abnormalities about the ileum and the ascending colon are of frequent occurrence and are developmental in origin.

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Discussion:

Dr. Hillman: We have had between 50 and 60 autopsies on infants and young children at the Post-Graduate Hospital during the past two years and I have been looking for adventitious peritoneal bands and have found in probably one third of the cases membranes which in the adult would be classified as abnormal peritoneal structures. Looking at the problem from the point of view of both anatomy and pathology, it would seem that in these very young children such a comparatively high percentage of cases in which these membranes are found would rather suggest that the affair is of a developmental character, and not inflammatory, as there are no other evidences of inflammation in the peritoneal cavity. Pericolic membranes, Lane's bands, or membranes about the hepatic flexure and elsewhere are often accompanied, in these children, by extremely lax mesenteric attachments, so that you have a loose mesocolon running from the cæcum right around to the rectum. In cases where the child has been marasmic we have noticed that this redundant condition of the mesocolon is marked. Whether the adventitious membranes are related to the laxity of the peritoneal ligaments I do not know, but it has struck me that these bands are of a developmental rather than of an inflammatory type.

FURTHER OBSERVATIONS OF THE THOMPSON-McFADDEN PELLAGRA COMMISSION UPON THE EPIDEMIOLOGY OF PELLAGRA

J. F. SILER, M.D., P. E. GARRISON, M.D., W. J. MACNEAL, M.D.

(*From the New York Post-Graduate Medical School and Hospital*)

ABSTRACT

Pellagra is a very definite pathologic entity characterized by typical lesions on the skin, by gastro-intestinal derangement and by disturbance of the nervous system. This disease is already very prevalent throughout the Southern United States, and cases have been observed occasionally in nearly every state in the Union. In Spartanburg County, South Carolina, we have been able to gather data concerning about 800 cases, the majority of which have originated in the last three years.

In Spartanburg County pellagra attacks all classes of the population but more especially the women and children living in the cotton mill villages. Pellagra morbidity in males between the ages of 15 and 45 is very low.

A statistical study of the food used in six of these mill villages, including about 5,000 persons, failed to reveal any consistent relationship between the use of any particular food and the occurrence of pellagra. A somewhat similar statistical study of the location of domicile of old cases of pellagra in relation to domicile of the remaining population in these mill villages has shown that new cases of pellagra developed almost exclusively in persons living in the same house with such antecedent cases or next door to them. In other words the disease spread from a preceding or antecedent case as a center, a phenomenon which can be, in our opinion, satisfactorily explained only by assuming that pellagra is an infectious disease. Apparently it is not readily transmitted to any considerable distance.

So far as we have observed in our field studies, pellagra spreads most readily in communities using unscreened surface privies. In those portions of the city of Spartanburg equipped with a water carriage system of sewage disposal, new cases of pellagra were few, and in two mill villages, completely equipped with water carriage sewer systems, it was impossible to find cases of pellagra which had certainly originated there. Individuals suffering from pellagra contracted elsewhere were not lacking in these communities.

(Lantern slides were shown, illustrating the clinical manifestations of the disease and the results of the investigations of the Commission as to the etiology of the condition.)

STUDY OF FERMENT INHIBITING SUBSTANCES IN TUBERCULOUS CASEATED MATERIAL

J. W. JOBLING, M.D., AND W. F. PETERSEN, M.D.

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University)

Caseation in tuberculosis is a form of coagulation necrosis in which the dead tissues rarely undergo autolysis, except as a result of secondary infection. Syphilis is the only infectious disease presenting a similar condition. In other instances of coagulation necrosis the dead tissues are soon removed by means of autolysis and phagocytosis. It appears, then, that substances having the property of preventing autolysis must be present in syphilitic and tuberculous tissues. This view is confirmed by the work of Schmoll and Auclair.

In the course of some work on ferments and ferment inhibiting substances, we observed that sodium soaps of the unsaturated fatty acids inhibit the action of trypsin and leucoprotease; that the activity of the soaps as ferment inhibiting agents is dependent upon the degree of unsaturation of the fatty acids; and that saturation of the acid with iodine causes a loss of this property.

The soaps of the unsaturated fatty acids tested do not have this influence on ferments.

These results led us to study the influence of the ether-soluble substances of tubercle bacilli on proteolysis. It has been shown by numerous investigators that the ether-soluble substances constitute 25–35 per cent. by weight of the bacilli, and of these extracts about 17 per cent. are composed of fatty acids. This study demonstrated that tubercle bacilli contain unsaturated fatty acids which, when saponified, have the property of inhibiting the action of trypsin and leucoprotease. According to their iodine value these soaps are more active than those tested previously and are dependent for their activity on the presence of unsaturated carbon bonds. Saturation of the soaps with iodine destroys their inhibiting action.

The above results led us to study tuberculous caseous material to find out if ferments and ferment inhibiting substances were present. The caseous material used in the study was obtained from tuberculous lymph glands and from several cases of rapidly progressing caseous pneumonia. In the experiments trypsin was used as the ferment and casein as the substrate. After the final incubation, the mixtures were made definitely acid with a solution composed of 10 per cent. acetic acid and 20 per cent. sodium chloride. The tubes were then placed in boiling water 5–10 minutes to coagulate the protein, and the contents afterwards filtered through kaolin. The total incoagulable nitrogen was determined according to the method recommended by Folin.

CASEOUS LYMPH GLANDS

The caseous matter was spread in thin layers on glass plates and dried at temperatures below 45° C. After being dried the material was ground in a mortar to a fine powder. The material was examined to find out if ferments and ferment-inhibiting substances were present and also for evidences of autolysis. To determine the extent of autolysis, a flask containing 50 c.c. of the emulsion was placed in the incubator and left there five days. Every day 5 c.c. were removed and the amount of incoagulable nitrogen was determined. The incoagulable nitrogen obtained in 5 c.c. of the emulsion on the fifth day was practically the same as that obtained on the first day. The freshly prepared emulsion was neutral in reaction, but became slightly acid after the second day, thus favoring the action of ferments active in a neutral medium and those active in acid medium. The results of this experiment indicate that ferments are not present in caseous material or that their activity is suppressed.

We obtained somewhat different results in a similar experiment conducted with caseous material from a lymph gland which had become secondarily infected. Autolysis occurred to a more marked degree in an alkaline reaction but was much more active in an acid reaction.

The lack of autolysis in the uninfected caseous material might have been due to the presence of soaps, and we therefore attempted to rule out this factor. Two methods were used to determine the presence of undestroyed ferments. In the first, the whole emulsion was treated with an excess of alcohol to which a small amount of hydrochloric acid had been added. Treated in this manner the ferments and protein were precipitated, and the fatty acids liberated by the hydrochloric acid were made soluble in the acid alcohol. The precipitate was then washed with alcohol and ether, and dried. The powder thus obtained was made into an emulsion with water, a portion made acid and another alkaline, and placed in the incubator for several days. The presence of ferments would have been shown by the degree of autolysis that occurred. In the second method, the emulsion was first incubated twenty-four hours in an alkaline solution in order to get the ferments and soaps into solution. It was then centrifuged and the supernatant fluid treated as was the whole emulsion. In this instance the powder was made into an emulsion and tested against casein. In neither case were we able to demonstrate the presence of ferments.

We believe the results of these experiments justify us in concluding that undestroyed ferments are rarely present in caseous lymph glands which have not become secondarily infected. Since practically all cells contain intracellular ferments, we must assume that substances were present which prevented autolysis. Our previous work indicated that these substances were probably soaps, therefore our next experiments were planned to determine if this were the case. A fresh 1 per cent. emulsion was prepared and kept at room temperature for several hours. During this period it was shaken frequently. It was then centrifuged until it became clear. The supernatant fluid was now removed, made strongly alkaline with sodium hydrate and the flask containing it placed in boiling water for several minutes. The fatty acids were then liberated with hydrochloric acid, taken up in ether and resaponified. The last step was repeated in order to free the soaps from unsaponifiable matter. The soaps were then tested for their enzyme inhibiting action. They were mixed in various dilutions with trypsin and placed in the incubator at 37° C. for thirty minutes. The casein was then added and the mixture placed in the incubator again for about two hours. After the final incubation the total incoagulable nitrogen was determined according to Folin's method. It was found that 3 c.c. of the emulsion, representing the soap contained in 0.03 gm. of caseous matter, was sufficient to inhibit the action of the ferment. In the tube containing 2 c.c. the digestion was only 25 per cent. of that obtained in the control tube containing no soaps.

The results obtained with the caseous material taken from a lymph gland which had become secondarily infected differ from those just described. Similar experiments were conducted with this material, which was semi-fluid in consistence, and ferments active in alkaline and acid reactions were demonstrated. While autolysis occurred in both acid and alkaline reactions, it was more marked in the acid reaction, indicating that soaps were still present. With this possibility in mind we decided to study the influence of iodine on the whole emulsion to see if it would accelerate autolysis. We have already shown that treating the soaps of the unsaturated fatty acids with iodine destroys their ferment inhibiting action, and it is therefore of interest to determine if similar results can be obtained with the native mixtures of protein and soaps.

The emulsion was divided into two portions, one of which was used as a control. To the other was added a mixture of iodine and iodide of potassium. After standing for several hours, the excess of iodine was removed by repeated extractions with chloroform. A third flask containing the washed precipitate was also used. To each of these flasks trypsin was added.

The results of this experiment show that in the flask containing the unstriated emulsion, soaps were not present in sufficient quantity to inhibit the action of the trypsin, but that digestion in a similar fraction treated with iodine was as great at the end of twenty-four hours as that obtained in the first flask after ninety-six hours. In the third flask the soaps had evidently been removed to a large extent, though digestion in the last seventy-two hours was not equal to that observed in the iodized fraction.

Experiments similar to those which have been described were conducted with material from the lungs of caseous pneumonia. It was found that ferments active in alkaline and acid media were present in the freshly prepared emulsion, but that the soaps inhibited the action of those active in an alkaline reaction. These ferments became active only after the soaps had been removed. Soaps were present in small amounts, but when isolated they were active as anti-enzymes. Iodine was effective here also in destroying the action of the soap.

In addition to the extractives of the tubercle bacilli which we have been discussing, lipoids derived from dead cells are also present in caseous matter. Neumann and others state that an alkaline solution of lecithin inhibits tryptic activity, but Kurt Meyer using a 1 per cent. solution was unable to confirm these observations.

In view of the uncertainty of the action of the lipoids we made a few experiments to see what influence they would have on enzyme activity. For these experiments lecithin, cuorin and cholesterin were used. The solutions were prepared by mixing 1 gm. of the lipoid with 100 c.c. of a 1 per cent. solution of an inactive preparation of sodium oleate. This sodium oleate preparation was one in which the acid had become saturated, thereby rendering it inactive as an inhibiting agent. By suspending the lipoids in an inactive soap solution we hoped to duplicate the physical condition present in the control containing the soap prepared from tubercle bacilli.

In addition to the preparations mentioned above, there was also tested in this experiment a soap prepared by saponifying the acetone insoluble fraction of the extractives of the tubercle bacilli. This soap is apparently much less active than that prepared from the acetone soluble fraction.

All the lipoids when tested in this manner appear to have some inhibiting action, particularly the lecithin which is most active. This experiment indicates that lipoids in the tissues, particularly in the presence of soaps, may aid in preventing autolysis, but their activity is much less than that demonstrated for the soaps.

DISCUSSION

We believe that we are now in a position to understand more clearly the processes leading to caseation in tuberculosis. With the lodging of the bacilli there is death of some of the cells due to the action of the toxins, and a proliferation of the neighboring cells. Under other conditions the death of a small number of cells would be followed at once by their removal, either through the action of phagocytes or by autolysis; but here the phagocytes are absent, and the action of the enzymes is prevented by the presence of the soaps of the unsaturated fatty acids derived from the tubercle bacilli. The anemia caused by the occlusion of the blood vessels plays an important part, as the lack of fluid prevents the dilution and subsequent absorption of the inhibiting substances. As the local process spreads slowly, the reaction at the periphery of the area prevents the entrance of fluids, and thus the caseous area gradually increases in size as the bacilli invade and cause the death of the surrounding tissues.

In conditions such as those present in caseous pneumonia, there is primarily an exudate composed of desquamated cells, leucocytes and fibrin, which soon become caseous. Before caseation is complete in the zone of gelatinous pneumonia, there is not the anemia due to occlusion of vessels that is seen in the more chronic processes, and we must conclude that the presence of caseation is due to substances inhibiting the action of the ferments present in the inflammatory exudate. An important factor in the acute process under discussion is the immense number of bacilli present. Under these conditions it is not difficult to imagine a corresponding increase in the amount of inhibiting substances, an increase sufficient to neutralize the ferments liberated by the disintegration of leucocytes and other cells. Caseous areas may persist for a long time without undergoing absorption. The rapid softening of these areas subsequently to secondary infections is probably due not only to the entrance of fresh ferments but also to the fluid accompanying these conditions, the fluid acting as a diluent, and aiding the removal of the inhibiting substances. Soaps if present must exist in very minute quantities in the bacilli. They are probably formed from the neutral fat of the bacilli. The lipases hydrolize the fats, and the acids are then saponified. Klotz and Bergell have demonstrated the presence of lipases in tuberculous pus and lymphocytes, while Wells and Corper found the ferment present in the tubercle bacilli.

According to Sata most of the fat present in caseous matter is found around the periphery of the area. This may be due to either a wandering in from the outside or, more probably, to synthesis from the fatty acids liberated during the disintegration of the cells and of the tubercle bacilli. Our experiments indicate that soaps of the unsaturated fatty acids obtained from tubercle bacilli are the active agents in preventing autolysis, and the identification of these acids will probably aid in determining the origin of the neutral fat found in caseous areas.

In our work on the ferment inhibiting substances present in tubercle bacilli we found that iodine combined with the unsaturated fatty acids obtained from the bacilli and neutralized their ferment inhibiting properties. We also emphasized the importance of this observation in connection with a specific chemotherapeutic agent for tuberculosis. In our present work we have shown that iodine exerts a similar action on the ferment inhibiting substances present in caseous matter. Thus the iodine may serve another purpose by aiding in bringing about solution and absorption of the caseous matter, and exposing the bacilli, which are otherwise more or less inaccessible, to the influence of the therapeutic agent.

It is a well known clinical observation that iodides cause tubercle bacilli to appear in the sputum of patients with pulmonary tuberculosis, though they were previously absent. This phenomenon is probably due to the neutralization of the action of the ferment inhibiting substances by the iodine. Subsequent to this neutralization, ferment action ensues, with liberation of the bacilli and their appearance in the sputum.

The autolysis that occurs in the center of large anemic infarcts and the lack of autolysis at the periphery can be explained in the same manner. At the periphery of the infarct the fatty acids which are liberated following death of the cells are saponified by the alkalies present in the tissues and by those coming from the surrounding fluids. The soaps thus formed inhibit proteolysis until the phagocytes, invading the periphery, open up channels through which sufficient fluid can enter to wash out or dilute the soaps so that autolysis can proceed. The phagocytes of course also take part in the removal of the dead tissues. In the center of the infarct the alkalies are soon used up and the tissues then become acid in reaction on account of the excess of fatty acids. Wiener found that autolytic enzymes act best in an acid medium, and this is to be expected since soaps are inactive as inhibiting agents under these conditions.

CONCLUSIONS

(1) Caseous matter obtained from lymph glands which have not become secondarily infected contains substances which inhibit enzyme activity. These substances consist chiefly of soaps of the unsaturated fatty acids.

(2) The inhibiting substances are present in relatively smaller amounts when the caseous matter has become secondarily infected. This is probably due to the dilution and washing out of the soaps.

(3) Ferments are either entirely absent or present in very small amounts, unless the caseous matter has become secondarily infected.

(4) Caseous material from the lungs contains smaller amounts of the inhibiting substances. This may be due to the acuteness of the process, not permitting an accumulation of the soaps, or to binding of the soaps with the ferments.

(5) Ferments are present in caseous pneumonia. In the whole emulsion the ferments are less active in an alkaline than in an acid reaction; but removal of the soaps shows that those active in an alkaline reaction are also present in considerable amounts.

(6) The previous treatment with iodine of caseous matter from both lymph glands and lungs increases the action of the trypsin.

Discussion:

Dr. Norris: I would merely like to remind the Society of the classical experiments along similar lines about ten or twelve years ago. Dr. Prudden showed that tracheal inoculation of tubercle bacilli produced a cheesy tuberculosis without suppuration. The suppuration or cavity formation occurred only when the trachea was subsequently inoculated with pyogenic cocci. Dr. Jobling's experiments have revealed to us the more intimate details of these complicated processes.

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Dr. A. M. Pappenheimer, *President*

REPORT OF A CASE OF PERIARTERITIS NODOSA

A. R. LAMB, M.D.

(*Pathological Laboratory, Presbyterian Hospital*)

Last year I reported briefly to the Society a case of periarteritis nodosa and showed the microscopic lesions of the disease. As there were no macroscopic nodules in that case the organs were not shown.

During the past month we have been fortunate enough to see a second case of this rare disease at the Presbyterian Hospital and in this instance it is possible to show the gross lesions as well as the microscopic.

The patient, a young girl ten years old, was admitted to the surgical side of the hospital January 18, 1914, with a history of having been taken ill nine days previously with sudden severe pain in the epigastrium. Five years previously she had had a less severe similar attack. Until admission to the hospital the pain continued intermittently and was associated with vomiting, fever headache and sore throat. On admission she seemed acutely ill. The throat was quite red. The abdomen was distentded and showed general resistance but no true rigidity. It was tender over all. While not perfectly typical, the diagnosis of appendicitis was considered probable, especially in view of a leucocyte count of 33,100 with 93 per cent. of polymorphonuclear leucocytes and a temperature of 102.6°.

An operation was accordingly performed soon after admission. The peritoneum was found perfectly normal. The appendix was removed and found to show no gross change. Exploration disclosed no cause for the symptoms.

Following the operation she continued to run a temperature between 102° and 103° and showed signs of inflammation with fluid in various joints so that it was thought for a time that she was suffering from acute rheumatic fever. The abdomen continued distended but was not tender. The urine showed a trace of albumin, a few casts and she developed a little œdema. Leucocytes continued high.

On January 28 she was transferred to the medical side where it was thought that she was suffering from sepsis following a streptococcus sore throat, or that she had had an undiscovered scarlet fever with a subsequent nephritis. On the medical side she had a temperature varying between 99° and 103.2°, a pulse of between 74 and 120. There was constantly a marked leucocytosis, the highest being 66,000 with 95 per cent. of polymorphonuclears. Three blood cultures were sterile, one of these being plated in ascitic glucose agar. The urine was generally diminished in quantity and showed a trace of albumin and casts. There was a slight œdema. For a time she seemed improved except for a recurrence of the paroxysmal abdominal pain and tenderness. She died quite unexpectedly on February 14, apparently from cardiac failure.

The autopsy was performed two and a half hours after death. The body was strikingly pale and there was a suggestion of puffiness about the face and slight œdema of the lower extremities. There were no subcutaneous nodules. The abdomen contained about 300 c.c. of clear fluid. The peritoneum was smooth and glistening except for a few small shreds of fibrin over the small intestine. There was a slight excess of fluid in the pericardium and about 100 c.c. in each pleural cavity. The lungs appeared normal. The heart weighed 320 gm. The branches of the coronary arteries presented the remarkable picture which you can see in the gross specimen. There were numerous, thickly placed, nodular thickenings, some of which showed small overlying petechial hæmorrhages. One or two of the nodules were definite aneurysmal dilatations filled with thrombi. Scarcely a single branch seems pervious. Scattered here and there in the heart muscles were dirty yellow patches. The cavities and valves of the heart showed no change. The large vessels were normal. The liver showed nothing more than some slight increase in the connective tissue about the portal vessels. The árteries seemed a little thickened in these regions but no definite nodules could be made out. The kidneys were most unusual. The surface was coarsely irregular, mottled red and opaque greyish yellow in color with a sprinkling of small yellowish points. The raised portions were red in color and often ran together in map-like patterns, apparently representing hæmorrhagic infarcts. On section, the above appearance is seen to extend into the cortex and medulla and the vessels are found to be thickened and to show tiny nodules. The spleen weighed 160 gm. The other organs presented nothing of interest. No gross nodules were found except those mentioned on the heart and kidneys. Permission could not be obtained for an examination of the nervous system, joints, or muscles of the extremities.

On microscopical examination, the characteristic lesions of periarteritis nodosa were found in practically all the organs and tissues.

A complete report of this case with the one shown last year will appear later in *The Archives of Internal Medicine.*

Discussion:

DR. PAPPENHEIMER: Dr. Lamb has been fortunate in having the opportunity to study two striking examples of this rare disease. He came to the second case with a prepared mind, and was able to make a more detailed bacteriological study than has heretofore been attempted.

PATHOLOGICAL FINDINGS IN THE INFECTIOUS EAR DISEASE OF RODENTS

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Laboratory workers and breeders of laboratory animals have long noticed that certain rodents—notably rabbits, white rats and white mice—at times develop peculiar symptoms especially in regard to locomotion and balance. These animals persistently hold their heads turned to one side, walk in a circle to one or the other side, easily fall to one side and have difficulty in regaining their normal upright position. The condition seems to be endemic in most laboratories where many of these animals are kept. So far I have not observed this condition in guinea-pigs.

CLINICAL PICTURE

The animal first becomes quiet and sluggish in movements. Later the head is held in a stiff position to one side or the other, rotated on its long axis with one ear held lower than the other, and usually the nose points in the direction of the lower ear. When the animal moves, the head position is more marked and he moves in a circle of greater or less diameter in the direction to which the head is turned. When the condition is advanced the animal has great difficulty in maintaining its normal crouching position. At the first attempt to move the animal falls toward the side to which its head points, and then proceeds to rotate rapidly in the same direction around the long axis of the body, rolling across the floor until stopped by some object. When a rat or mouse with this disease is picked up by the tip of the tail he does not remain still as the normal animal does but immediately starts to throw himself around in the same direction in which he falls until he is rapidly rotating around his tail at right angles to it. It is not possible to say whether these animals are deaf in one ear or not. No nystagmus is observed.

The movements of these animals are very similar to those of the dogs in which one cerebellar hemisphere and the labyrinthine root on the same side are destroyed. In these animals Thomas reports that the head is turned toward the side of the lesion and the rotations around the long axis of the body occur toward the same side.

MATERIAL

RAT. I. Duration of disease unknown. Head held rotated and turned to the left. In moving goes in a circle to left. When placed on back, he rotates around the long body axis to the left three or four times before coming to rest. Picked up by tail he turns to left.

Killed by chloroform and both ears and brain removed for section. Brain stained by Weigert Pal showed nothing. Right ear normal. There is pus in left ear, filling middle and internal ear with complete destructions of ossicles.

RABBIT I. Duration two days. Symptoms here were referable to the left side. Head turned and rotated to left. Falls to left. Gait in a circle to left. Rotation around body axis to left. Killed by chloroform. Brain not used. Ears opened. Right ear normal. Left ear full of thick, white, stringy, almost gelatinous pus. Smears made of this pus showed many Gram-positive streptococci with some diplococci which appeared to be encapsulated,—probably peculiar forms of streptococci.

RABBIT II. Symptoms were referable to the left side and were the same as in rabbit I. He became progressively worse, head 90° to left, and at length he was unable to make the slightest movement without immediately falling to the left and rotating in this direction around the long body axis many times until fatigued. After two weeks he died. On autopsy the right ear was found to be free while the left was full of the same pus as described above.

The brain stained according to the Marchi method shows a complete degeneration of the eighth nerve on the left side, both cochlear and vestibular branches of the nerve being destroyed.

Rat II. Duration unknown. Symptoms were referable to the left side as in the others. There were no unusual symptoms during life which distinguished this rat from the others suffering from this disease. However he did appear to be sicker than the others and less capable of locomotion and maintaining balance.

On autopsy an abscess was seen in the left cerebellar lobe with some purulent exudate on the left side of the medulla. There was no pus free in the cranial cavity nor were there any signs of perforation upward from the ear.

In examining sections of the hind brain in this animal one sees that the entire region of the left eighth nerve is included in a mass of exudate and necrosis. The infection has beyond doubt ascended the nerve and involved the dorsal part of the medulla, especially the region occupied by the nucleus vestibularis triangularis. From this point the process has spread probably along the restiform body—into the left lobe of the cerebellum where in sections nearer the frontal region we see it as a partly walled-in abscess which, at one point, has broken through into the fourth ventricle. Very little pus is to be seen free in the ventricle. The involvement of the triangular nucleus is well seen in one section. Here there is a rather thick layer of necrosis in the floor of the fourth ventricle—on the left side only—which stops abruptly at the median raphe.

DISCUSSION

The pathology of the condition becomes clear, when we consider the anatomy of the auditory nerve. Each part—cochlear or vestibular—of the eighth nerve has the same structure as the spinal nerves. Of the vestibular branch, the end organs are the ciliated cells of the saccule and semi-circular canals. The vestibular ganglion, or Scarpa's ganglion, lying on the perforated plate at the bottom of the internal auditory canal is the analogue of the post nerve root ganglia of the spinal nerves containing bipolar cells. The vestibular root represents the posterior spinal roots. It enters the postero-lateral part of the medulla both dorsal and ventral to the restiform body and the fibers terminate in the nucleus triangularis descendens and Deiter's nucleus and form tracts which run down to the cord and up into the cerebellum.

Of the cochlear branch, the cells of the organ of Corti constitute the end organ. The spinal ganglion lying in the spinal plate contains the bipolar cells. The cochlear root enters the lateral surface of the medulla ventral to the vestibular root, forming the corpus trapezoideus which decussates and terminates in the superior olive of the other side.

In these animals we have a purulent otitis affecting all the ear structures. Undoubtedly, the infection travels up the nerves and involves and destroys the two ganglia. This is followed by degeneration of the nerve roots to their central termination. The symptoms produced are thus attributable to the destruction of both branches of the eighth nerve.

The cerebellar abscess in Rat II represents only a further extension of the process, a further ascension of the infection up the eighth nerve roots to involve in an abscess those portions of the medulla and cerebellum which were rendered functionally inactive by the ascending degeneration of the nerve as seen in Rabbit II. It is rather remarkable that the great cerebellar destruction and brain pressure shown in Rat II did not occasion more severe symptoms.

Discussion:

Dr. Zinsser: The disease which Dr. Casamajor has described is not uncommon among white rats. About five years ago it occurred among the white rats we were using at St. Luke's Hospital and the lesions appearing in the ears of these rats corresponded with those which Dr. Casamajor has described. However, the bacteriology of this disease does not seem to be uniform, since Dr. Casamajor described finding a streptococcus in the pus. In our cases, which Dr. Echeverria examined, there was present a long slender bacillus which, as far as we could determine its species, was closely related to the organism described in books on diseases of animals as the bacillus necrosus.

TUBERCULOUS ULCER OF VULVA

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The specimen was removed by operation by Dr. Edwin B. Cragin, through whose courtesy it is presented. The patient, a married woman of 35 years, came to the Hospital last January with a history of no previous illness or operation. She had two children. She was admitted for ulceration of the vulva. This had begun two months previously with swelling of the left labium which broke and discharged. One month later the right labium became similarly affected. She had no pain, no bleeding from ulcer and no cough, expectoration or loss of weight.

Physical examination showed râles and prolonged expiration over right upper lobe. Abdomen negative. Inguinal lymph nodes enlarged. Over posterior part of vulva and about anus there was a large excavated ulcer on both labia. Edges were undermined and granulations unhealthy looking. Uterus and adnexæ normal. Temperature 100° F., pulse 112. Wassermann reaction was negative.

A section from the edge of the ulcer excised for diagnosis showed tubercles with areas of coagulative necrosis. A fair number of tubercle bacilli could be demonstrated in the tissue. At the operation all the ulcerated areas were excised together with enough healthy tissue anteriorly to the mons and posteriorly to the anus to give satisfactory flaps for closing the denuded areas. During the past six weeks the temperature has ranged from 100° in the morning to 102° or 103° at night, and the signs in the lungs have become more extensive. The wound broke down in portions and an attempt at fulguration was followed by a severe chill and rise of temperature to 105°.

Tuberculosis in this region seems rather uncommon. Dr. Cragin said that he had seen the condition before. Howard Kelly in his gynecology describes a case of tuberculosis of the vestibule and says that it is a rare affection of the external genitals. Allbutt's *Gynecology* gives references to a half dozen cases. All these observers consider it a secondary condition and usually associated with phthisis pulmonalis. We believe that in this case the involvement of the vulva was secondary to the lung condition.

A CASE OF INFLUENZAL AND TUBERCULOUS MENINGITIS

H. K. FABER, M.D.

(*Babies Hospital*)

Tuberculous meningitis with a secondary meningeal infection appears to be a rare event. Paisseau and Tixier reviewing the subject in 1909 collected nine cases from the literature and reported one of their own, the complicating organisms mentioned being *B. coli, meningococcus, M. tetragenus* and an unclassified Gram-negative diplococcus. Guinon and Grenet, and Dupérié report secondary infections with meningococcus, and the autopsy records of The Babies' Hospital show one case each of pneumococcus and of streptococcus infection complicating meningeal tuberculosis. Meningitis in which the influenza bacillus is associated with another pathogenic organism occasionally occurs. Staphylococcus, streptococcus, pneumococcus and, in one case, meningococcus have been found in such cases.

In tuberculous lungs the influenza bacillus is not at all uncommon. It is mentioned by Petrushky as a particularly severe form of secondary infection and has been found by several investigators. It has been grown from tuberculous lungs 22 times in The Babies' Hospital.

The coincidence of influenzal and tuberculous meningitis has not hitherto been described.

CASE REPORT

S. S., male, Italian, five months old. Admitted to The Babies' Hospital, February 3, 1914, for convulsions. The mother has had a cough for the last two months. Two weeks ago the baby was weaned. Since then he has not thrived, but has had no other untoward symptoms until the night of January 31, when he had a general convulsion lasting three to five minutes, following which the head became retracted and has remained so since. The next day he had seven convulsions, the following day ten, and the next day, on which he was admitted, he had one more. He has been restless and irritable, and has slept little. There has been no vomiting.

Physical Examination. A poorly nourished infant who looks acutely ill. He is irritable. The neck is stiff, and the head retracted.

Head. Fontanelle bulges very slightly, but is not tense.

Chest. A few coarse râles were heard behind the angles of the right scapula. (Later, signs of consolidation were found in the upper left lobe).

Abdomen. The liver and spleen are felt just below the costal margin.

Lymph glands. A few palpable nodes in neck, axillæ and groin.

Extremities. Legs slightly spastic and semiflexed. There is no Kernig.

Reflexes. Knee jerks exaggerated.

The tuberculin skin test next day was strongly positive. The blood showed 19,000 leucocytes, 37 per cent. polynuclear and 63 per cent. lymphocytes. 18 c.c. of clear cerebro-spinal fluid removed at this time (February 4) showed 145 cells per c.mm., 92 per cent. of which were lymphocytes. The pressure was normal. The next day another specimen was obtained and was seen to contain some small Gram-negative bacilli. A culture on blood agar was unfortunately overgrown with *Staphylococcus albus*, and in view of the character of the fluid and the positive Von Pirquet no further attempt at culture was made. A blood culture was made and showed a very heavy growth of the influenza bacillus, also unfortunately contaminated. On February 6 a choroidal tubercle was found in the right fundus. On February 14 tubercle bacilli were found in the spinal fluid, which still remained clear. 45 c.c. under pressure were removed. There were 375 cells per c.mm. and these were practically all lymphocytes. The case was thus proved to be one of tuberculous meningitis and the finding of other organisms was regarded as accidental.

On February 17, the influenza bacillus was found in a culture from the bronchial secretion.

From the time of admission to that of death (February 18) the patient was in opisthotonos and had many general convulsions. No unilateral symptoms were noted.

AUTOPSY

The important autopsy findings were: Caseous tuberculosis of the left bronchial lymph-nodes (one of these nodes led to caseous areas ending in a large cavity under the pleura anteriorly) and a disseminated miliary tuberculosis involving practically all the viscera. The pia everywhere on the surface of the cerebrum, cerebellum, pons and medulla was studded with small miliary tubercles. In the interpeduncular space and over the pons and medulla was a fairly abundant fibrino-purulent exudate. The sulci over the left cortex were filled with a yellowish gray pus. This was most abundant over the frontal and anterior parietal regions but extended almost to the posterior pole and also to the inferior surface along the Sylvian fissure. The ventricles were normal and contained clear fluid under normal pressure. There was some yellowish pus in the right middle ear.

Smears from the meningeal pus showed influenza bacilli which were also seen, together with pneumococci, in the smear from the right ear. Cultures from the pus on the left cortex gave a pure growth of *B. influenzæ*. Injected intravenously into a guinea-pig and a rabbit it killed both in twenty-four hours, and was recovered from their blood. Cultures from small areas of broncho-pneumonia near the cavity in the left lung showed a pure growth of *B. influenzæ*.

DISCUSSION

Certain unusual features of this case may be pointed out. The predominance of lymphocytes in the blood and spinal fluid is unusual in a case of influenzal meningitis. The extreme spasticity and frequent general convulsions over a long period are both unusual in tuberculous meningitis.

The temperature curve was also unusual for tuberculous meningitis. The temperature two weeks before death rose to 104° and 105°, and pursued an intermittent course bearing close resemblance to that often seen in our cases of respiratory influenza. In fact it may be assumed, in view of the positive sputum culture, that the character of the fever was due to the influenza bacillus.

The purulent infection was evidently recent and it may be that it had developed after the last lumbar puncture which was four days before death. If this was the case we must assume that the Pfeiffer's bacilli, circulating in the blood two weeks before death, had not as yet attacked the cortical pia and did so only as a terminal event.

Discussion:

Dr. Pappenheimer: I should like to ask if Dr. Faber has observed anything characteristic about the color of the exudate in influenzal infection which gives one a hint as to its etiology.

Dr. Faber: The color is not characteristic. There is not the greenish color seen in the pneumococcus lesions.

A SIMPLE METHOD OF INDUCING SPIROCHÆTA PALLIDA TO GROW IN FLUID MEDIA

H. ZINSSER, M.D., AND J. G. HOPKINS, M.D.

(Department of Bacteriology, College of Physicians and Surgeons, Columbia University)

The method which I wish to describe is presented to the Society because Dr. Hopkins and I have found it useful and we trust it may prove equally so to others who are working with the *Spirochæta pallida*. It represents, in substance, an adaptation of the Noguchi technique. We employ "U" tubes, which are easily made in the laboratory, of large glass tubing, plugged and sterilized in the usual way. When we transfer strains of *Spirochæta pallida* we run into these tubes a very small amount of agar-ascitic fluid mixture, just enough to fill the bridge which connects the two arms of the "U." We then put a piece of sterile rabbit's kidney or rat kidney (we have found rat tissue quite useful for pallida cultivation) into each arm of the tube, pushing it down until it rests upon the hardened agar in the connecting arm. We then inoculate with sp. pallida on one side only and into the inoculated arm pour agar mixed with ascitic fluid in the usual way. After the agar has hardened throughout, we pour a mixture of about half and half ascitic fluid and sterile salt solution into the other arm, seal both sides with a little sterile albolene and incubate. The spirochæta grow through from the agar arm to the ascitic fluid arm appearing about the tissue into the fluid medium sometimes as early as the fifth day. The growth of the spirochæta on the fluid side is usually quite profuse. So far however we have not been able to purify cultures by this method. We hope that the method will prove useful for this purpose; it seems to be only a question of transplanting at a time before the bacteria have come through. In the cultures we have examined early so far, on the fifth day, the spirochæta on the fluid side have been more profuse than bacteria.

OBSERVATIONS ON PRECIPITIN FORMATION BY BOILED PROTEINS

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The study of the effects of heating upon various antigens was carried out in connection with the agglutinins and typhoid bacilli by Joos, Eisenberg and Volk, Kraus and Joachim, and Scheller. Without going into the details of these rather complicated investigations we may summarize the results by saying that the immunization of an animal with typhoid bacilli heated above 62° C. produces an immune serum which, as Paltauf has expressed it, is "umfänglicher" than one which is produced with the living organisms. By this is meant particularly the fact that the immune serum produced with living bacilli will agglutinate highly the living organisms but will have a relatively weaker action upon heated cultures. The sera of animals immunized with bacilli heated to 60° to 62° C., however, agglutinate both heated and unheated bacteria equally well. A similar observation was made by Kolle and Gottschlich who advised the use of heated cholera cultures for the purpose of rapidly producing potent agglutinating sera.

The production of precipitins with heated antigens has a certain amount of practical importance, in that the precipitin reaction is being used extensively in meat inspection, where the material which is to be examined has often been heated. Obermeyer and Pick,[1] studying this problem, found that the precipitin produced with unheated beef serum does not react with boiled beef serum but that the sera of animals immunized with beef serum which had been boiled for a short time ("kurz aufgekocht") contained a precipitin which reacted not only with the native beef serum but also with boiled serum and a whole row of split products which gave no reaction to normal precipitin. The "heat-precipitin" so produced, then, would also fall into the definition of Paltauf in being "umfänglicher" than that produced with the unheated antigen. More recent work by Schmidt[1] seems to show that heating the serum protein to 70° for as long as 30 to 60 minutes influences its precipitability by "native precipitin" only in so far as it diminishes the delicacy of the reaction by 10 to 30 per cent., and that heating to 90° for as long as an hour does not render it entirely non-precipitable, so that proteins so treated may yet be detectable by ordinary specific precipitins produced by the injection of unheated serum, though the delicacy of the reaction is lessened. Boiling, however, in Schmidt's experience rendered the proteins non-precipitable by the "native precipitins." Fornet and Müller[2] disagree with Schmidt in this particular in that they believe that even boiled protein can be detected with native precipitin although the delicacy of the reaction is reduced by about one tenth. Schmidt later recommended the immunization of animals with serum protein heated to 70°, a procedure which yielded an antiserum that gave strong and practically useful reactions with its specific antigen, even when this had been boiled. He later describes a heat-alkali precipitin which he obtained by immunizing rabbits with blood serum which had been heated for 30 minutes at 70° C. with the addition of weak sodium hydrate solution. This heat-alkali precipitin gave specific reactions with homologous protein that had been heated to 70° to 100°, and even with insolubly coagulated protein after this had been boiled with sodium hydrate. This "heat-alkali precipitin" did not react with native protein. Fornet and Müller claim that precipitin can be produced in animals with sera heated up to 86° C. but that temperatures above this destroy its precipitin-inducing properties. This is contrary to the experience of Obermeyer and Pick as well as our own.

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[1] *Wien. klin. Woch.*, 1906.

The practical utilization of any of these methods must naturally depend upon the specificity obtained by the precipitins so produced. In Obermeyer and Pick's original investigations they found that precipitin produced with heated serum is as strictly specific as that induced by native serum. This also has been the experience of Schmidt. Fornet and Müller, on the other hand, report that the precipitins produced by them with heated muscle protein were not as strictly specific as those produced with unheated protein in that the former gave precipitates not only with homologous protein solutions but with foreign proteins in moderate concentration as well. Apparently for practical purposes the specificity of the 70° precipitins of Schmidt is sufficient, with careful control, to yield accurate results in the determination of boiled protein. According to Fornet and Müller native precipitin can be used though with less delicacy. Since there is some difference of opinion as to the result obtained when animals are immunized with boiled protein we have carried out a number of tests which are illustrated in the following protocol.

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[1] *Biochem. Zeitschr.*, 14, 1908; *Zeitschr. f. Imm.*, vol. 13, 1912.

[2] *Zeitschr. f. Hyg.*, vol. 46, 1910.

TABLE II

Experiments on Cocto-precipitin (March 23, 1913)

Cross Titrations; dilutions of sera in salt solution boiled 5 minutes; precipitated with antisera produced by injections with similarly boiled material.

The readings here indicated were taken by "ring" test at the end of 30 minutes and on the following morning by settling.

Dilution	Beef-serum *vs.* Anti-beef Precipitin	Beef-serum *vs.* Anti-dog Precipitin	Beef-serum *vs.* Anti-sheep Precipitin	Dog-serum *vs.* Anti-dog Precipitin	Dog-serum *vs.* Anti-beef Precipitin	Dog-serum *vs.* Anti-sheep Precipitin	Sheep-serum *vs.* Anti-sheep Precipitin	Sheep-serum *vs.* Anti-dog Precipitin	Sheep-serum *vs.* Anti-beef Precipitin
1:20	+	+	+	++	— —	+	++	++	+
1:50	+++	+	+++	++	— —	++	+++	+	+++
1:100	+++	+	++	+	— —	+	++	+	++
1:500	++	— —	+	+	— —	+	+	— —	— —
1:1000	+	— —		+	— —	+	+	— —	— —
Controls of boiled serum alone:									
1:20	— —			— —			— —		
1:50	— —			— —			— —		
Serum control									

Note: These controls were necessitated by the fact that the boiled serum suspensions were themselves turbid and occasionally showed slight settling on standing.

We have done a large number of experiments of this kind with variable results, the lack of specificity being unequal in individual cases, and it must be stated that, in most cases, the strongest reactions were obtained when homologous antigen and antiserum were used. But powerful reactions were also obtained in many cases with boiled sera other than those used for immunization.

We may summarize the results of these experiments and of other similar ones, as follows: When animals are immunized with boiled proteins, precipitins are formed which react powerfully with the homologous boiled protein but the specificity is to a large extent destroyed in that precipitates, almost as powerful, can be produced with boiled proteins other than those used for immunization. It is interesting to note also that here as in the case of normal precipitins an excess of antigen inhibits the reaction.

THE RENAL EPITHELIUM IN EXPERIMENTAL HYDRONEPHROSIS

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The gross and microscopical changes in the kidney following abrupt obstruction of the ureter have been studied recently by Ponfick, Winkler, Suzuki, Boetzel and others and in the two latter papers the method of vital staining has been applied to determine the changes in the epithelial cells. Before the appearance of these papers the work to be described was begun with rats, using the innocuous trypan blue as the staining reagent. This stains the tissues in a few hours and disappears after eight or ten days. It is preferable to the carmine used by Suzuki in that it is not poisonous and our experiments differed from those of Boetzel, who used tolidin blue, in that the injections were made repeatedly and long before the death of the animal while his were made only a few hours ante mortem.

Attention was especially directed to the influence of obstruction (1) upon the secretion, (2) in dilating and distorting renal structures and (3) in producing destructive changes and atrophy in the renal epithelium. It was thought that alterations in the epithelial cells might be followed especially well if the Altmann's stain and the injection of trypan blue were used to make distinct the specific structural components of their protoplasm. It is known that in the normal kidney the trypan blue appears in the protoplasm of the epithelial cells of the convoluted tubules in fine granules. In the proximal part of the convoluted tubule the granules are dense, compact, and intensely stained and grouped about the nucleus. In the medial portion they are less distinct and less densely stained and in the distal part there is only a scattered fine granular staining. The distribution of the rods and granules stainable by the Altmann method is well given in Suzuki's monograph. It is seen at once that the granules stained by trypan blue do not correspond with those stained by the Altmann method but exist side by side with them in the same cell. It may be that were these particular granules not stained with the blue they would show the Altmann stain and be indistinguishable from the rest, but the combination of the two stains shows at least that even if this be so there is a specialization among the Altmann granules which allows some of them to assume the blue dye. On the other hand, it is possible that the blue stained granules are quite distinct from the group of Altmann granules.

In these experiments the right ureter was ligated in rats and on the same day and every ten days afterward, 3 to 5 c.c. of a 1 per cent. solution of trypan blue in Ringer's solution was injected subcutaneously. The animals were killed after periods ranging from forty-eight hours to one hundred and one days. Detailed protocols will be given in a more extended publication and it may be said here that the obstructed kidney cells steadily, becoming one third larger than the opposite organ after forty-eight hours and after thirty days the size of a large walnut. After one hundred and one days it appears as a cyst containing as much as 36 c.c. of blue fluid with a transparent wall only ½ mm. thick. That secretion is possible even when great dilatation of the kidney has occurred was shown by the aspiration of the fluid from the sac after seventeen days. In a very short time it again became tightly distended.

The gradual reduction of the kidney substance is evident, as Ponfick pointed out, even though the kidney becomes greatly enlarged. Dilatation of the tubules could be observed in the earlier days up to the tenth or twelfth day but after that they appeared to collapse and many of them assumed the form of solid strands of epithelium. It is remarkable that these changes do not affect all the tubules uniformly for dilated canals are seen side by side with normal or collapsed ones. Nor is the progression of the change perfectly regular, for sometimes more extensive dilatation is found after a few days than in another animal after a much longer time. Still the general progress of the alterations is as described. From about six days onward wandering cells and fibroblasts appear between the tubules and in the later stages the new growth of conective tissue becomes very conspicuous.

The condition of the Altmann granules corresponded also somewhat irregularly with the progress of the lesion, for in even the later stages occasional tubules can be found in which they are still intact. After six days' obstruction some tubules are observed in which the epithelial cells contain the blue stain in the form of droplets or as a diffuse staining. This is evidence of their injury since the normal cells store this dye in the form of sharply stained granules. The proportion in which this abnormal storing of the dye and staining occurs, increases with the age of the lesion and in the very late stages no cell shows clear cut granules but only diffuse staining and droplet formation. Since the blue is absorbed from even the diseased cells after about ten days and excreted by the other kidney the method of continuous injection is not open to the objection that the blue found in diseased cells may be that which was taken up when those cells were in better condition and retained there.

When the injection of the blue dye is made only a day or two before the death of the animal it is found that the fluid in the dilated pelvis becomes colored even after thirty days' ligation when the kidney is reduced to a thin sac. This may be taken as evidence of the continuance of secretion even into this late stage. This is further shown by the continued distension of the sac and its failure in the rat to contract into a smaller structure as it does in other animals.

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DR. A. M. PAPPENHEIMER, *President*

THE USE OF CHOLESTERIN ANTIGEN IN THE WASSERMANN REACTION

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A method of preparing a standard antigen for the Wassermann reaction is given in the Yale number of the *Archives of Internal Medicine.*[1] The advantage of the method is that an antigen may be prepared at any time that gives constant results in the reading of the reaction. This antigen is an alcoholic extract of guinea-pig heart half saturated with cholesterin; the reason for the addition of this latter substance at the present time is unknown, but it seems to stableize the reaction.

Table A shows 5 antigens of this type tested with 7 positive sera.

Table B shows one of these tested against duplicate sera.

Of late there have been a number of articles criticizing the addition of cholesterin to the syphilitic antigens, most of the authors claiming that it renders the antigen too sensitive. As a result of something more than five thousand routine tests[1] comparing the acetone insoluble antigen with this cholesterin antigen, it was found more constant and more reliable if certain fundamental conditions be observed in using it. Some workers recently have recommended the use of 1 or 1½ units of complement instead of the 2 units as used in the original Wassermann method, in order to render the test more delicate. The individuals recommending this modification can have but a slight knowledge of serum work for they seem not to realize that Wassermann adopted the two units of complement for a very definite and, to all serological workers, a well-known reason. The complement is thermolabile, and its exposure to a temperature of 37½° C. for one hour decreases its activity. Therefore, if but one unit of complement is used some is destroyed during the binding period and so a partial inhibition will occur without any reaction between antibody and antigen.

The cholesterin antigen must be used with between 2 and 3 units of complement and even 4 units will only reduce the reading slightly. The presence of a native hemolysin may be ignored entirely as it makes no difference if there be two or ten units of amboceptor present, if the sheep cells have been sensitized with hemolysin before they are added to the serum complement antigen mixture. These facts may be seen in Table I. Again, with cholesterin antigen it is unnecessary to make use of the ice-box incubation of the serum complement antigen system as the cholesterin seems to increase the velocity of the reaction. The rule in this laboratory is to incubate the complement serum antigen mixture in a water bath for not more than 50 minutes at 35–36° C. As has been pointed out,[2] six different dilutions of the antigen are used for testing each serum. The greatest amount of antigen used is 1/10 of a milligram, with 1/10 of a c.c. of the serum, plus 2 to 3 units of complement. The second antigen tube contains .08 mg., the third .06, the fourth .04, the fifth .02 and the sixth .009 mg. In this way we have a series of tubes with a constant amount of serum and a decreasing amount of antigen and if we let 0 represent complete hemolysis, 1 marked hemolysis, 2 slight hemolysis and 3 no hemolysis, we have a possible numerical series of 0 to 18 depending upon the amount of inhibition of hemolysis in this series of tubes. For complete inhibition in all 6 tubes would give $3 \times 6 = 18$.

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[1] All patients over three days in the hospital are supposed to have a Wassermann test. Fifty to one hundred tests are performed each day, five days of the week.

[2] *Arch. of Internal Medicine*, 1914, vol. XIII, no. 5.

In the series of over 5,000 cases only one half of 1 per cent. have given complete inhibition in the .009 mg. tube. If too little serum is obtained to use in the six antigen tubes and one or two controls, we can reverse the procedure and vary the amount of serum, keeping the antigen constant at .1 mg. in each tube. The results of such a procedure may be seen in Table II and it shows well the close agreement in the final readings of the two variations. The amount of antigen .1 mg. is $^1/_5$ the anti-complementary dose with the complement diluted 1 to 8 and using .5 c.c. This may be seen in Table III. We do not find anti-complementary substances in the serum if it be poured off the clot within 12 hours after collection and centrifuged free of red cells.

With this cholesterin antigen the serum must be inactivated. The constancy of the unit readings was demonstrated in the previous paper[1] and in Table IV there are shown various readings on the same patient obtained at different times. A child was admitted to the hospital as a case of amaurotic family idiocy. This child gave negative Wassermann reactions but in its appearance suggested a case of congenital lues. The blood from the father (B. S., a Hebrew, 28 years of age) was found to have 15 units. He did not accept this report and had his serum taken by a private physician who sent it to the Board of Health; it was reported a very strong positive. Not satisfied with this, and unknown to ourselves, he came to our dispensary and his blood was again sent in to this laboratory where it was reported again 15 units. He then went to a large private hospital in this city where he had his Wassermann taken again and this time it was reported negative. For some unknown reason, he was dissatisfied with this report and returned to our dispensary where the blood was once more taken and sent to the laboratory and reported back 15 units. At the time these readings were made the previous reactions were unknown to us. He came to the laboratory a week after the last report, thinking that it was another institution, to have another test made. He claimed that he had never been sick and had never had a chance to contract the disease. On questioning him closely it was found that he had not been in robust health for some years, that his wife had not been well since she had married him and that the child with amaurotic family idiocy was the youngest, the elder one (six years of age) having always been a sickly and poorly nourished infant. He was requested to bring his wife and eldest child to the laboratory, which he did. Blood was obtained from all three and the result of the tests is shown in Table IV. The child presented the appearance of a case of mild congenital lues with slightly pegged teeth. The mother stated that she had had one miscarriage at six months and one birth of a dead child at full term, that she suffers continually from headaches and is ill nourished. The husband complained for the first time of his mouth being sore; his tongue showed several typical mucous patches. With the acetone insoluble antigen the child showed a weak positive, and the mother was negative. In Table IV the reactions are with the cholesterin antigen.

The clinical diagnosis of syphilis may be extremely difficult and we now know that there are many cases of latent or mild lues in which the disease is not suspected and in many cases no so-called primary or secondary lesions are observed and these patients may give negative Wassermanns and show no symptoms leading one to suspect a diseased condition clinically. The court of last resort as to the value of the Wassermann reaction must be the post-mortem examination. In this hospital the positive Wassermanns, even when only 1 or 2 units during life have been constantly associated at post-mortem with pathological lesions which have been recognized for years as being syphilitic. There have, on the other hand, been a certain number of cases

TABLE A

Comparison of Five Different Antigens with Seven Positive Sera

	Serum A							Serum B							Serum C							Serum D						
	Antigens							Antigens							Antigens							Antigens						
	X	1.	.8	.6	.4	.2	.09	X	1.	.8	.6	.4	.2	.09	X	1.	.8	.6	.4	.2	.09	X	1.	.8	.6	.4	.2	.09
Antigen I	0	3	3	3	3	2	0	0	3	3	3	3	3	2	0	3	3	3	3	0	0	0	3	3	3	3	0	0
Antigen II	0	3	3	3	3	3	0	0	3	3	3	3	3	1	0	3	3	3	3	1	0	0	3	3	3	2	0	0
Antigen III	0	3	3	3	3	1	0	0	3	3	3	3	3	3	0	3	3	3	3	0	0	0	3	3	3	3	0	0
Antigen IV	0	3	3	3	3	2	0	0	3	3	3	3	3	2	0	3	3	3	3	3	0	0	3	3	3	2	0	0
Antigen V	0	3	3	3	3	3	0	0	3	3	3	3	3	2	0	3	3	3	3	1	0	0	3	3	3	1	0	0
	High 15 Low 13							High 18 Low 16							High 15 Low 12							High 12 Low 11						

	Serum E							Serum F							Serum G						
	Antigens							Antigens							Antigens						
	X	1.	.8	.6	.4	.2	.09	X	1.	.8	.6	.4	.2	.09	X	1.	.8	.6	.4	.2	.09
Antigen I	0	3	2	1	0	0	0	0	3	3	3	3	3	0	0	3	0	0	0	0	0
Antigen II	0	3	3	3	0	0	0	0	3	3	3	3	3	0	0	2	0	0	0	0	0
Antigen III	0	3	3	2	0	0	0	0	3	3	3	3	2	0	0	3	1	0	0	0	0
Antigen IV	0	3	3	1	0	0	0	0	3	3	3	3	3	0	0	3	0	0	0	0	0
Antigen V	0	3	3	3	0	0	0	0	3	3	3	3	1	0	0	2	1	0	0	0	0
	High 9 Low 7							High 15 Low 13							High 4 Low 2						

Forty other sera were negative. 0 = complete hemolysis; 1 = marked hemolysis; 2 = slight hemolysis; 3 = no hemolysis; X = control.

showing syphilitic lesions which have given during life a negative Wassermann. Until there is obtained a series of one thousand or more autopsies with Wassermann reactions taken during life and correlated with the autopsy findings, it is merely guess work to state the absolute value of this reaction. We must, it seems, revise a great many of our conceptions in regard to lues. The more we study it in the light of the Wassermann reaction, the more closely does it resemble tuberculosis in its protean mani-

TABLE B

Comparison of Unknown Duplicate Tests

Serum	Control	.10	.08	.06	.04	.02	.009	Units of serum	Units in spinal fluid
J. K.	0	3	3	3	3	3	0	15	15
J. K.	0	3	3	3	3	3	0	15	
T. R.	0	3	3	3	3	2	0	14	
T. R.	0	3	3	3	3	3	0	15	
P. C.	0	3	3	3	3	3	2	17	15
P. C.	0	3	3	3	3	3	2	17	
J. L.	0	3	3	3	3	3	2	17	
J. L.	0	3	3	3	3	3	1	16	
M.	0	3	3	3	0	0	0	9	
M.	0	3	3	2	0	0	0	8	

Twenty-three other cases were 0. 0 = complete hemolysis; 1 = marked hemolysis; 2 = slight hemolysis; 3 = no hemolysis.

TABLE I

Comparison of Varying Amounts of Complement and Amboceptor on a Positive Wassermann

A	*B*	*C*	*D*	*E*	*O*	Antigen Tubes					
						.10	.08	.06	.04	.02	.009
.1	2	2	1	16	0	3	3	3	3	3	1
.1	4	2	1	14	0	3	3	3	3	2	0
.1	6	2	1	8	0	3	3	2	0	0	0
.1	2	4	1	16	0	3	3	3	3	3	1
.1	4	4	1	13	0	3	3	3	3	1	0
.1	6	4	1	7	0	3	3	1	0	0	0
.1	2	6	1	16	0	3	3	3	3	3	1
.1	4	6	1	13	0	3	3	3	3	1	0
.1	6	6	1	10	0	3	3	3	1	0	0
	2	2	1	0	0	0	0	0	0	0	0

A = amount of patient's serum in 1 c.c.; B = units of complement; C = units of amboceptor; D = units of sheep red blood cells; E = total number of units; O = control tube; no antigen.

TABLE II

Comparison of Positive Sera with Serum Constant, Antigen Variable, and Antigen Constant, Serum Variable

		0	1	2	3	4	5	6	T.
58	*A*	0	3	3	3	3	3	0	15
	B	0	3	3	3	3	3	0	15
62	*A*	0	3	3	3	2	0	0	11
	B	0	3	3	3	1	0	0	10
66	*A*	0	3	3	3	3	3	0	15
	B	0	3	3	3	3	3	1	16
67	*A*	0	3	3	3	3	3	0	15
	B	0	3	3	3	3	3	1	16
70	*A*	0	3	3	3	3	3	0	15
	B	0	3	3	3	3	3	0	15
72	*A*	0	3	3	3	2	1	0	12
	B	0	3	3	3	3	2	0	14
73	*A*	0	3	3	3	2	0	0	11
	B	0	3	3	3	3	1	0	13
75	*A*	0	3	3	3	3	1	0	13
	B	0	3	3	3	3	3	0	15

A = serum constant, .1 c.c. antigen variable, 1 = .1 mg.; 2 = .08 mg.; 3 = .06 mg.; 4 = .04 mg.; 5 = .02 mg.; 6 = .009 mg.

B = antigen constant, .1 mg. serum variable, 1 = .1 c.c.; 2 = .08 c.c.; 3 = .06 c.c.; 4 = .04 c.c.; 5 = .02 c.c.; 6 = .009 c.c.

O = control tube, 1 c.c. serum, no antigen.

TABLE III

Anticomplementary Titration

A	Amounts of Antigen in Milligrams									
	.1	.2	.3	.4	.5	.6	.7	.8	.9	1
.4 c.c.	0	0	0	0	1	1	2	2	3	3
.5 c.c.	0	0	0	0	0	1	1	2	2	3
.6 c.c.	0	0	0	0	0	0	1	1	1	2

After 1 hour at 37° C. 1 unit of sheep red cells sensitized with 2 units of amboceptor were added. Column A = amounts of 1 to 8 complement solution.

Complement titer with 2 units of amboceptor + 1 unit of non-sensitized sheep red cells.

Amounts in c.c. of 1 to 8 complement:

.5 c.c.	.4 c.c.	.3 c.c.	.2 c.c.	.1 c.c.
0	0	0	0	1

TABLE IV

B.S.	Jan. 28, 1913	15 units
B.S.	Feb. 18, 1913	15 units
B.S.	Mar. 11, 1913	15 units
B.S.	Mar. 3, 1913	15 units
Wife	Mar. 3, 1913	2 units
Child	Mar. 3, 1913	11 units

festations. Also, we must bear in mind that there are, without doubt, variations in the virulence of different strains of spirochæta and that there are variations in the resistance of individuals to the spirochæta. Not until a very large series of cases has been studied in the routine manner described will we be able to follow this disease in all its manifestations.

In conclusion, we must remain in a neutral position in the face of weak positive reactions and in justice to the patient we should give a provocative dose of salvarsan or neosalvarsan and see whether or not the reaction is increased. In this hospital we have in several cases found it necessary to give two or even three and four full doses before being able to obtain a positive reaction in known syphilitics. In view of the possibility of these cases developing syphilitic lesions of the parenchymatous type we must be on our guard and watch such patients very closely, since after the development of paresis there is little hope in specific therapy.

Under specific treatment the reaction slowly disappears and the serum from week to week shows a gradual drop in the number of reacting units. The reaction disappears with this cholesterin antigen much more slowly than with any of the other antigens. The statement made recently in the *British Medical Journal* that a positive reaction with this antigen only means that the patient has had syphilis and does not indicate a still active process is, in our opinion, unjustified by the facts and our observations.

THE USE OF CHOLESTERIN ANTIGEN IN THE WASSERMANN REACTION

J. G. HOPKINS AND J. B. ZIMMERMANN

As the subject of cholesterin antigen was to be brought before the society this evening we thought it would be of interest to report our experience with these preparations. We first made a trial of them as they seemed to offer a means of detecting reactions in serum which failed to react with older types of extract. In order to test their sensitiveness and reliability we used them in parallel with lipoid antigens in a series of routine Wassermann reactions at St. Luke's Hospital.

The technique used was practically that of the original Wassermann reaction except that half quantities were used throughout, *i. e.,* 0.1 c.c. of patient's serum, 0.05 c.c. of guinea-pig serum, 0.5 c.c. of 5 per cent. sheep cells, sensitized with two minimal haemolytic doses of immune rabbit serum. The first incubation was carried on for one hour at 37° C. and the readings were made one hour after adding the sensitized cells. The control antigens were prepared by Noguchi's method from the acetone insoluble fraction of beef heart extract, as our previous experience had convinced us of the reliability of such extracts.

After some preliminary trials with antigen prepared by Sachs' method of adding varying amounts of a 1 per cent cholesterin solution to crude alcoholic extracts we adopted the method recommended by Walker and Swift—adding 0.4 per cent. dry cholesterin to the alcoholic extract and after 24 hours filtering off the undissolved residue. We used, however, guinea-pig heart instead of human. We also diluted the extract for use in a different manner, adding the salt solution drop by drop to the alcoholic stock solution. This gives a turbid emulsion which is more anticomplementary but also more active as an antigen than the clearer emulsions obtained by adding the alcoholic solution slowly to the diluent.

We used these extracts in about 800 tests. After discarding duplicates and cases with unsatisfactory histories 539 remained which we tabulated as follows according to the clinical diagnosis. In order to eliminate the personal factor as far as possible we have called all doubtful reactions negative and have included as positive only those which showed less than 10 per cent. hæmolysis as judged without a scale after an hour's incubation in the second stage of the reaction.

Condition Blood Serum	No. of Cases	Lipoid Antigen, Positive Reaction, Per Cent.	Cholesterin Antigen. Positive Reaction, Per Cent.
Primary syphilis	10	60	70
Secondary syphilis	25	84	100
Tertiary syphilis	99	59	80
Congenital syphilis	9	60	80
Tabes dorsalis	20	45	65
General paresis	4	100	100
Latent or cured	44	38	66
Non-syphilitic	264	1.5	9
Spinal Fluid			
Tabes dorsalis	19	42	52
General paresis	3	100	100
Tertiary syphilis	9	33	22
Non-syphilitic	19	0	5

In other words of these 525 specimens 455 reacted alike with both antigens. Two (a spinal fluid from a case of cerebro-spinal syphilis and one from a case of tabes) gave a positive result with the lipoid and a negative one with the cholesterin antigen. Sixty-eight that were negative with the lipoid were positive with the cholesterin antigen. Of these cases, 46 were clinically syphilitic or gave some history of syphilis and 22 were apparently non-syphilitic. Of these apparently false positive reactions four were from patients with carcinoma and one from a sarcoma case and three were from patients with pernicious anæmia. It is interesting that two other cases of pernicious anæmia gave doubtful reactions with the cholesterin antigens. One was a case of malaria; the other cases showed a wide variety of conditions. It is unlikely that their illness at the time had any connection with the reaction.

In view of the difficulty of obtaining accurate histories from many hospital patients it is impossible to say positively that all these cases were non-syphilitic. There were four cases which reacted with the lipoid antigen though negative clinically and from our experience with these extracts we feel certain that these patients had an unrecognized latent syphilis. It seems to us, however, highly improbable that all the twenty-two that reacted only with the cholesterin preparations were luetic. However, even if a few false positives are obtained these antigens give so much higher percentage of reactions in tertiary and latent syphilis that we believe they are of considerable value in cases which fail to react with less sensitive preparations. From our experience, however, it would not seem safe to make a positive diagnosis on a reaction with cholesterin antigen unless there is some clinical evidence in its support.

SALVARSAN TREATMENT OF SYPHILIS AS JUDGED BY THE WASSERMANN REACTION USING A CHOLESTERIN FORTIFIED ANTIGEN

E. G. STILLMAN, M.D.

(Pathological Department, Presbyterian Hospital)

The antigen used in doing these Wassermann reactions was an alcohol extract of guinea-pig heart to which 4 per cent. cholesterin was added, as recommended by Walker & Swift.[1]

During the year from February 1, 1913, to February 1, 1914, 47 cases of syphilis which gave a positive Wassermann reaction with this cholesterin antigen before treatment was begun, received two or more salvarsan or neosalvarsan injections.

In the dispensary cases, three primary cases after receiving 3, 7 and 11 salvarsan injections were rendered negative; a fourth case after 5 injections remained positive. In these cases there was some doubt of its being a recent infection as the lesion on the lip was not a typical hard chancre.

Of the five secondary cases one changed to negative after 10 salvarsan injections, one remained 2 + after 6 injections, while three, two of which received 6 and one 18 injections, remained 3 + positive.

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[1] Walker, I. C., and Swift, H. F., *Jour. Exper. Med.*, 1913, XVIII, 75

The proportion of positives among the tertiary cases is very high as out of 18 cases showing 3 + positive before treatment, 13 after receiving from 4 to 17 salvarsan injections remained 3 + positive; two cases dropped to 2 + after receiving 5 and 10 injections respectively and in only one instance was the Wassermann rendered negative after 14 salvarsan injections. Of the 19 hospital cases all in the tertiary stage giving a 3 + positive reaction, 18 which received from 2 to 13 salvarsan injections remained 3 + and one dropped to a 2 + after 5 salvarsan injections.

Discussion of papers by Drs. Field, Hopkins, Zimmermann, and Stillman on the Wassermann reaction:

Dr. Swift: I wish to emphasize the point brought out by Dr. Field, that it is necessary to have a very active hemolytic system in using cholesterin reinforced antigens. If only one unit of complement is used, one obtains many non-specific results. (Some recent condemnations of cholesterin reinforced antigens have been made by workers who used only one unit of complement in carrying out the reaction.) We have found it necessary to titrate the complement each day for the strength varies much, according to the season of the year and feeding of the guinea-pigs. We have been mostly interested in the use of these antigens in the diagnosis and treatment of patients with syphilis of the central nervous system. About 20 per cent. of these cases that gave a positive Wassermann reaction in the blood with the cholesterin heart antigen, gave negative reactions with liver antigens. Subsequent examination of their cerebrospinal fluid and antisyphilitic treatment proved them to be suffering from active syphilis. The Wassermann reaction disappears much more slowly under treatment when the cholesterin heart antigen is used than with the liver antigens. We have tested a large number of non-syphilitic cerebrospinal fluids, and, up to the present time, have found no non-specific reactions with the cerebrospinal fluid even when large quantities of the fluid were used in performing the test.

Dr. Kaliski: I agree with Dr. Swift in the necessity of accurately titrating the various ingredients used in the tests. The accuracy of the results depends upon the quantitative relationship existing between the factors in the hemolytic system. In using the reinforced cholesterin-alcoholic extract it is of prime importance that the hemolytic system be very sensitive; at least a double hemolytic unit or dose of complement must be used if non-specific results would be avoided. On the other hand the presence of anti-sheep (natural) amboceptor in patients' serum must be taken into account, otherwise the hemolytic system may be too sensitive. Often (in about 50 per cent. of cases and especially in young children) as many as two units and occasionally more than two units of natural amboceptor are contained in 0.1 c.c. of inactivated serum. This excess of amboceptor, plus the usual two units used in the test, are often sufficient to make a weak or moderately positive reaction negative. This factor therefore must be taken into account in comparing statistics of various authors using various antigens. The avoidance of this error in the anti-human system is one of the reasons for its greater sensitiveness.

In my opinion a good lipoid antigen, prepared from beef heart, guinea-pig heart, or syphilitic liver, properly selected and tested for anticomplementary and hemolytic properties and used in one-fourth to one-sixth the beginning binding dose cannot be exceeded in sensitiveness by a reinforced antigen. In my experience of more than five years with clinical material easily controlled, I have not seen non-specific results. Thus, with the lipoid antigen even slight degrees of inhibition of hemolysis can be viewed with suspicion, while with the reinforced antigens it is unwise to regard anything except a complete inhibition of hemolysis as indicative of syphilis.

It is of extreme importance that only such antigen be used for the diagnosis of lues as will give absolute specific results. From a consideration of the figure put in evidence by the readers of the papers the reinforced cholesterin antigen is not such an antigen, even if used in not greater concentration than 0.4 per cent. My experience with a suspension of this strength has been such as to lead me to believe that the greater sensitiveness of the reinforced antigen may possibly be due to the summation of slight anticomplementary factors in the serum and antigen. Therefore, it is wiser to discard a poor alcoholic extract than to attempt to reinforce it with cholesterin. A properly lipoid extract offers a reliable, sensitive, specific substitute.

Dr. Ottenberg: Can Dr. Field give us any idea as to how the cholesterin acts to make alcoholic extracts, which at the beginning had different antigenic values, equal each other. The cholesterin itself is not antigenic. It is well recognized that extracts of different beef hearts and even extracts of different sets of 50 or 100 guinea-pig hearts have often very different antigenic as well as anticomplementary effects. How does the cholesterin act to standardize these?

I must agree with Dr. Kaliski that in some patients there is a large amount of natural anti-sheep amboceptor and it is obvious that in such cases if sensitized blood cells are added weak positive Wassermann reactions appear to be negative. Several authorities have suggested different ways of circumventing this difficulty. Dr. Field did not state whether he paid any attention to it.

Dr. Larkin: I would like to state that I agree entirely with what Dr. Field has said and absolutely disagree with Dr. Hopkins. In a very large service on the Island we have been convinced of the reliability of the cholesterin antigen. We have been able to verify our conclusions at autopsy in a number of positive cases.

Dr. Warren: I should like to ask Dr. Field what results he has obtained in cases of pernicious anemia. Our reactions have been negative except in one case in which at autopsy an aneurysm was found.

Dr. Field: In regard to Dr. Kaliski, I took it for granted that everyone realized that the reagents were all titrated, and in regard to the natural sheep amboceptor in the patient's serum I would say that everyone who has any knowledge of serum work knows that it may be ignored when the red cells are sensitized before being added to the serum complement antigen mixture. I have not had any difficulty in the presence of sheep amboceptor in my antigens.

It is easily seen from Dr. Hopkins' table that his antigen complement has been used in quantities of two units and, as I stated, with the use of cholesterin antigen we should use 2½ to 3 units. We have run more than 5,000 cases with both the acetone insoluble antigen and the alcoholic extract of guinea-pig heart plus cholesterin to one half saturation.

I believe Dr. Longcope reported last year that in the routine examination of the patients in the medical service of Presbyterian Hospital he obtained between 25 to 30 per cent. positive reactions. With the use of the cholesterin antigen we have found in Bellevue that on the medical service 25 per cent. of our cases give positive reactions. The figures in regard to the positive reactions as they occur in different clinical conditions are interesting. Among our pneumonias we find 24 per cent. positive; among our typhoids on the other hand only 2 per cent. have given positive reactions; whereas in the case of aneurisms since we have been using this cholesterin antigen we have obtained 99 per cent. positive.

In regard to Dr. Warren's question on pernicious anemia I would state that we have not obtained positive reactions except in one case that gave a history of a primary untreated sore.

Dr. Hopkins: I did not undertake the Wassermann reactions without first testing the hemolytic systems to be used that day. We used two units of amboceptor and one of complement, *i. e.*, .4 to .5 c.c. of a 1 to 9 dilution as determined by the preliminary titration.

In regard to percentages, we had too few cases to make the percentage figures accurate. The 5 per cent. of the cases of spinal fluid in which we got one non-specific fixation means only one case. This was a case of tuberculous meningitis and we were unable to exclude absolutely congenital syphilis. We laid more emphasis on the tests on blood serum which were sufficient in number to make the percentages significant. In regard to the amount of antigen used, one quarter of the amount did not cause any inhibition of hemolysis. We found the cholesterin antigen unstable compared with lipoid. With old cholesterin preparations there were many non-specific results. When the antigen becomes too anti-complementary it is time to get another.

FACTORS OF RESISTANCE TO HETEROPLASTIC TISSUE GRAFTING

JAMES B. MURPHY, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York)

It has been shown that the chick embryo offers suitable conditions for the growth of implanted tissues, whether these be embryonic or adult of the same species or of a foreign one.[1] The chick at about the time of hatching develops a defensive mechanism against the tissue of foreign species.[2] This resistance can be supplied to the embryo in the early stages if grafts of adult spleen or bone marrow are implanted.[3] Under these conditions the embryo exhibits the same resistance to foreign tissue as does the adult, and presents the same histological manifestations about the graft. Furthermore, the same tissues, spleen and bone marrow, when grafted into an embryo with an established and growing rat tumor, bring about a retrogression and absorption of the foreign tissue. Other adult tissues do not supply this power to the embryo.

If the deductions from these experiments are correct, it should be possible to effect heterotransplantation in the adult by lessening the activity of the lymphoid tissue. Rats treated with Röntgen rays for a period long enough to cause extensive destruction of the lymphoid elements of the spleen, bone marrow, and lymph glands, no longer possess a defensive mechanism against heteroplastic tissue.[4] For instance, the Ehrlich mouse sarcoma will grow in a rat so treated for as long as 19 days. By transferring the mouse sarcoma to freshly X-rayed rats as soon as the host shows signs of regeneration of the protective system, the mouse tumor will continue to grow in rats for as long as 37 days and with proper handling perhaps indefinitely.

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[1] Murphy, Jas. B., *Jour. Exper. Med.*, 1913, XVII, 482.

[2] Murphy, Jas. B., *Jour. Exper. Med.*, 1914, XIX, 181.

[3] Murphy, Jas. B., *Jour. Am. Med. Assn.*, 1914, LXII, 199; *Jour. Exper. Med.*, 1914, XIX, 513.

[4] Murphy, Jas. B., *Jour. Am. Med. Assn.*, 1914, LXII, 1459.

Discussion:

Dr. Lambert: There are several questions which I should like to ask. Has the extract or juice of adult spleen or bone marrow any effect when injected with the foreign tissue? Is it possible that the inhibiting action of spleen may be due to the survival and growth of its cells in the embryo, whereas the cells of other organs do not survive?

The growth for at least thirty-seven days of a mouse tumor in rats treated with X-rays is very significant since the usual period of growth is less than ten days. Several years ago we observed in two experiments active growth for seventeen days. In each case the rat was united with a mouse (parabiosis), and the increased growth of the mouse tumor in the rat under these conditions was regarded as favoring Ehrlich's hypothesis.

I should like for Dr. Murphy to give us briefly his interpretation of his results in regard to the general question as to why heteroplastic grafts do not succeed. Bashford's cytotoxin theory has something to support it in that definite cytotoxic substances *are* formed when an animal is treated with the cells of a foreign species, as we have shown in tissue culture studies. But in those experiments it was necessary to use relatively large doses of the foreign tissue. It should be stated that Dr. Murphy's results are not necessarily opposed to the theory of protective antibody formation, since the mononuclear cells, whose importance he has demonstrated, may protect through the formation of antibodies.

Dr. Jobling: I should like to ask if the point of insertion of the spleen tissue has any influence on the result. Does the tissue exert a local action, or is the effect definitely a systemic one?

Dr. Pappenheimer: Is it possible to bring this into any relation with the lipolytic action of mononuclear leucocytes?

Dr. Murphy: In answer to Dr. Lambert's questions, I will add that spleen extract has been repeatedly tested and apparently fails to supply the embryo with the protection against foreign tissue. The grafts of kidney and connective tissue grow as well if not better in the embryo than the spleen grafts, therefore the explanation can not be sought in a competitive affinity for the food particles. As to explanations of these results, I do not care to go beyond the statement that by supplying adult lymphoid organs to the embryo it can be made resistant to heterotransplantation, and that an adult animal deprived of its lymphoid tissue no longer has a defensive mechanism against heterotransplantation.

In answer to Dr. Jobling, I do not know the source of the round-cell infiltration around a graft of foreign tissue in the embryo provided with adult lymphoid cells. They are present whether the spleen graft be near the foreign tissue or at the extreme pole of the egg. As to the mode of action of the lymphoid tissue, I have no direct proof.

TWO CASES OF TUBERCULOSIS OF UTERUS

E. G. STILLMAN

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The first case of tuberculosis of the uterus was described by Moyagne in 1744. Louis and Senn then described cases but after this the infection was lost sight of until Reynard reported two cases in 1831. Since this time a great many cases have been reported.

Uterine tuberculosis occurs once in about every hundred autopsies but is more frequent in cases already suffering from tuberculosis. Different observers report it as occurring in from 1 to 8½ per cent. of autopsies on tuberculous cases.

In rare instances tuberculosis of the uterus is apparently primary. Generally, however, there are other tuberculous foci in the body and in a large majority of cases the tubes are also involved. Occasionally, no other focus of tuberculosis can be found in the body. Gorowitz mentions a rare budding form of cervical tuberculosis which may be mistaken for sarcoma or carcinoma.

Dervaux mentions five possible modes of infection:—(1) primary ascending infection from vagina; (2) descending—from peritoneum and tubes; (3) arterial; (4) venous; (5) lymphatic.

The venous and lymphatic routes although possible must be very rare. Although tubercle bacilli have been demonstrated in sperm cells by Moffucci and Jaeckh this mode of infection is not likely. The vagina is very resistant to tubercle bacilli as Gorowitz has demonstrated by depositing without trauma pure culture of tubercle bacilli in the vagina of guinea-pigs and rabbits without producing any lesions. The bacteria might be introduced by instruments or soiled clothing. But even if a few bacilli did gain entrance in this way, as Williams remarks, the few minute tubercles would probably be thrown off with the menses. They could only get a permanent hold if they reached the tubes where they could remain unmolested.

A descending infection from the peritoneum is much more likely as the tubes are so frequently involved in peritoneal tuberculosis. Jani has even found tubercle bacilli in the lumen of a normal tube in a case with tuberculous ulcers of the intestines. Willliams, however, believes the commonest mode of infection to be by the blood. In proof of this he cites the fact that women who develop miliary tuberculosis in the puerperium have the greatest number of tubercles at the placental site. This mode of infection would also account for the cases in which the uterus is involved when there is no tuberculous focus in the intestines, peritoneum or mesenteric lymph glands. As in a number of cases the tuberculous process is most marked at the fimbriated extremity of the tube and at times is entirely limited to it, he considers that this is not an extension from the peritoneum but a hematogenous infection, the fimbriated extremity being so much more vascular than the other parts of the tube. The uterus may also be involved by a direct extension of the process from neighboring organs by a fistula, or by the perforation of tuberculous ulcers from an adherent intestine, rectum or bladder.

Kiwisch considers that the puerperium apparently acts as a predisposing cause for tuberculosis of the uterus. A number of cases of miliary tuberculosis of the uterus have occurred as a result of a general miliary tuberculosis in the puerperium or following abortion. In these cases the tubercles are most marked at the placental site.

The tuberculous process is almost always limited to the body of the uterus and rarely extends beyond the internal os to involve the cervix. In nearly all cases the initial lesion is in the form of miliary tuberculosis. At first the process is limited to the endometrium which is infiltrated by miliary tubercles which are situated just beneath the epithelium. These later become caseous, the epithelium disappears, and shallow ulcers studded with tubercles are seen. The most frequent stage of the process seen at autopsy, however, is the chronic diffuse tuberculous stage in which the entire cavity of the uterus is filled by caseous material. The jagged and irregular walls of the uterus are studded with tubercles in all stages of development. The uterus is often enlarged by the accumulation of the caseous material and by a hypertrophy of the muscularis. The wall of the uterus may be so weakened and destroyed that the uterus ruptures. In other cases, especially in old women, if the cervical canal is obliterated, the caseous material and secretions may continue to accumulate until a pyometra is formed.

CASE I

M. S.—Greek woman, 40 years old. Entered Dr. Janeway's service, Presbyterian Hospital, February 13, 1914.

Family History.—Negative.

Past History.—Has always been well until two years ago, when she began to have sharp abdominal pain. Although this pain was aggravated by eating, there was no vomiting or nausea. One year ago her abdomen was opened at Alexandria, but she did not know what was done. Following the operation the pain was somewhat relieved. Menstruation stopped two years ago, but she has had a slight amount of bleeding two or three times since.

Present Illness.—About three months ago she sailed from Alexandria. During the month of the voyage she was very seasick. Since landing she felt very weak and for the three weeks before admission has been unable to get out of bed. Her abdominal pain recurred more severely. She had some diarrhœa and had lost much weight.

Physical Examination.—Physical examination showed an emaciated elderly woman. The lungs and heart were negative. The abdomen was slightly distended and tender on deep palpation in the lower portion. No masses or signs of fluid were made out. The cervix was not movable and no masses were felt in the fornices. Patient gradually became weaker and died on February 14, 1914.

AUTOPSY

An autopsy was performed 27 hours post mortem. The body was greatly emaciated, and the abdomen was markedly distended. On making the preliminary incision much odorless gas escaped. Lying in the meshes and in the pocket-like spaces between the coils of intestines as well as freely in the abdominal cavity about 75 c.c. of a thin opaque yellow fluid was seen. The intestines were everywhere covered by fine flakes of fibrin, and were bound together by firm fibrous adhesions. There were two or three large caseous lymph nodes in the parietal peritoneum of the abdominal wall. Shining through the peritoneal covering of some of the loops of intestines pearly white nodules were seen. The intestinal coils lying in the pelvis were firmly bound together and adherent to the cæcum and appendix. In one place a pin-point perforation was found through which yellowish fluid could be pressed.

The left lung was bound down by fibrous adhesions and there were about 50 c.c. of reddish fluid in the pleural cavity. Scattered over the lung irregular, elevated, firm, grayish areas were seen with distinct tubercles about the periphery. On section irregular grayish areas whose central portions suggested beginning caseation but in which distinct miliary tubercles, more distinct away from the center, were seen irregularly scattered throughout the lung. The right lung presented a similar picture. The heart was normal. The liver appeared very fatty. The spleen showed a few pearly white nodules which were taken to be tubercles. The pancreas, stomach and adrenals were normal. The kidneys showed only cloudy swelling. There were a number of tuberculous ulcers in the jejunum and ileum which encircled three fourths of the intestine. These ulcers had entirely eaten away the mucosa and submucosa and their bases were formed of irregular granulation tissue. The site of the perforation was found to be in the center of an ulcer.

The *uterus* was retroverted and bound down by a few fibrous adhesions. The peritoneum covering the uterus was injected. The uterus measured 3 × 5 × 8 cm. and felt hard. On section the cavity of the uterus was found to be filled with caseous material averaging ½ cm. in thickness. The uterine muscle cut with a gritty sensation and on the cut surface the thickened vessels stood out prominently. The cervical canal was filled with a thick mucus. The cervix except for two lateral scars, was apparently normal. The tubes were tortuous and adherent. Their outer halves were greatly distended measuring 3½ cm. in diameter. On section the tubes were seen to be distended by caseous material.

On *microscopic examination* the lungs presented irregular, distinct and confluent grayish areas whose central portion was filled by caseous material. The periphery of these areas was formed by an irregular outline of discrete and conglomerate grayish tubercles. In sections stained for tubercle bacilli these were seen in great numbers. The liver presented an advanced stage of fatty infiltration and a number of miliary tubercles were seen. No tubercles were seen in the pancreas. The spleen showed miliary tubercles which tended to be in the periphery of the Malpighian bodies. The intestinal ulcers showed tubercles in the adjacent villi and others were present at their borders, in floor and in the submucous tissue.

The endometrium of the uterus had been entirely destroyed, no vestige of a gland being seen. Instead the uterine surface was covered by a caseous material which contained much nuclear dust and small wandering cells. There was no sharp line of demarcation between this caseous mass and the uterine muscle, but the caseous areas extended in an irregular way into the uterine musculature. A little further epithelioid cells, small mononuclear wandering cells and loose bundles of connective tissue cells as well as necrotic material and nuclear debris loosely filled up the space between the bands of fibrous tissue. Scattered through the uterine musculature, which was very fibrous, were small accumulations of mononuclear wandering cells and giant cells. Some of these areas appear as typical miliary tubercles with giant cells. The lumen of the Fallopian tubes were distended by a structureless homogeneous pinkish staining material. A few giant cells and many mono-nuclear cells were present in the edges of the caseous material. No remnants of mucous membrane were found, the caseous material being surrounded by the thickened fibrous walls of the tube.

CASE II[1]

E. V.—Aged 28. Entered Dr. Lambert's service at the Presbyterian Hospital, September 27, 1913.

Family History.—Negative.

Personal History.—Has had three children.

Past History.—Three years ago had pleurisy with effusion on left side. Two years ago had an attack of "rheumatism" in all her joints which left her with a bad elbow and knee. Night sweats began one year before admission. In March, 1913, the left leg was amputated and tuberculous tissue was excised from the left elbow joint.

Present Illness.—Began four months before admission with pain in left lumbar region.

Physical Examination.—Physical examination showed a boggy fluctuating swelling in the back in the left lumbar region. This cold abscess was incised but no tubercle bacilli were found. The patient developed a cough, râles appeared in her lungs and signs of fluid in the abdomen. She gradually became weaker, and the sinus continued to discharge. During the last week she was unable to retain nourishment because of vomiting.

AUTOPSY

Patient died on February 21, 1914, and an autopsy was held three hours post mortem. The body was that of a moderately well nourished white woman. The left elbow was apparently the seat of an old tuberculous process and several small sinuses discharged pus from an indurated scar over the olecranon. The left lung was bound down by firm fibrous adhesions. On section small irregular areas of consolidation were seen but there was no evidence of tuberculosis. The right lung was everywhere adherent by firm fibrous adhesions and the pleural cavity contained about 200 c.c. of clear amber fluid. There were a few pearly white nodules on the pleura of the right upper lobe and a small patch of these nodules on the diaphragmatic surface. On section, except for a small area of broncho-pneumonia in the upper lobe, the lung appeared normal. Except for a slight stenosis of the mitral orifice with some thickening of the valves, the heart appeared normal. The liver was firmly adherent to all neighboring organs. On separating these adhesions without difficulty the inner surface was found to be greenish and necrotic and about 300 c.c. of thick greenish purulent material escaped from the gall bladder. No stones were found. On section the liver was mottled by yellowish and reddish areas, while the central portion of some nodules suggested amyloid. The pancreas appeared normal. The spleen was bound down by firm fibrous adhesions and on section many areas of amyloid were seen. The surface of the left kidney was matted with green translucent and opaque granular areas. On section similar greenish and yellow areas were seen in the pyramids and cortex. The right kidney was similar to the left, but in addition there were three areas of caseation in the cortex about 15 mm. in diameter which extended into the papillæ. The mucous membrane of the pelvis was congested, roughened and apparently contained young tubercles. The bladder appeared normal. The left adrenal was round and nodular. The medulla was a mass of caseous material which extended into the cortex. The right adrenal was similar to the left.

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[1] I want to thank Dr. Coulter for permission to present his case.

The *uterus* was normal in size, and on section was seen to be filled by caseous material which invaded the walls for a short distance. The external os was soft, eroded in small areas, and showed small areas of caseation. Both Fallopian tubes contained caseous nodules and the tubes were held backward by delicate adhesions to the peritoneum. The extremities contained large caseous masses. The left ovary contained a small area of caseation but none was found in the right.

The eighth thoracic vertebra was surrounded by a "cold" abscess and the body of the vertebra was much softened. The lumbar and sacral vertebræ were extensively eroded and surrounded by large psoas abscesses.

Microscopic examination showed brown atrophy and cloudy swelling of the heart. The lungs showed a beginning broncho-pneumonia but no evidence of tuberculosis. The spleen showed an extensive amyloid degeneration which involved nearly every Malpighian body. There were no tubercles present. The liver showed an advanced stage of fatty infiltration, some amyloid change of the capillaries and an occasional small but definite tubercle with giant cells. The wall of the gall bladder was much thickened by fibrous tissue which extended into the liver in an irregular manner. There was no evidence of tuberculosis, the sections showing nothing more than chronic inflammation. The adrenals showed caseous and amyloid masses with only a few cortical cells which could be identified. The muscle bundles of the uterus were separated by a considerable amount of fibrous tissue. As one approached the lumen a dense wandering cell infiltration was seen and then large caseous areas with peripheral nuclear dust. The free margin was formed of a ragged pink necrotic material. No giant cells were seen. The tube showed a mass of caseous material surrounded by a fibrous tissue capsule in which were areas of wandering-celled infiltration. The vagina showed much wandering cell infiltration beneath the epithelium but no definite tubercles.

For literature see:

Williams, J. W., Johns Hopkins Hospital Reports, 1893, III, 85.
Murphy, J. B., President's Address, Chicago Surgical Society, October 19, 1903.
Routier, A., *Thèse de Paris,* 1909.
Gorovitz, M., *ibid.,* 1900.
Dervaux, H., *ibid.,* 1902.

Discussion:

Dr. Tytler: Last fall we had a case at the New York Hospital which is interesting as regards the mode of entrance. It was an extensive tuberculosis of the left kidney with a localized patch of tuberculosis at the fundus of the uterus. The tubes and peritoneum were quite free from tuberculosis and the path of infection was apparently by the vagina.

Dr. Field: We had a case at Bellevue Hospital which came to autopsy. It had been diagnosed as carcinoma of the uterus, but was found to be a case of tuberculosis. Only the uterus, vagina and rectum were involved. There was an ulceration through the rectum into the vagina at the junction of the cervix and body of the uterus, and it had spread through from there; towards the bladder the tubercles were fewer and smaller. There was nothing in the lungs or in any of the other organs.

Dr. Stillman: There was one case at the City Hospital in which at autopsy a tumor mass of the cervix was taken for carcinoma. Microscopic examination revealed its tuberculous nature. Cases of tuberculosis of the uterus may be more common than they are thought to be.

RECENT STUDIES IN THROMBO-ANGEITIS OBLITERANS

LEO BUERGER, M.D.

Since the year 1904 I have been collecting pathological material and clinical data on that interesting group of cases which have been described by the Germans under the name, "Endarteritis Obliterans," and for which in May, 1908, before the American Association of Physicians I proposed the term "Thrombo-angeitis Obliterans." At that time my deductions were based on a study of the vessels in nineteen amputated limbs, but, to-day, I am able to draw my conclusions from a much larger number, namely forty amputated limbs, two amputated forearms and some twenty-five extirpated veins from eighteen cases of thrombo-angeitis afflicted with migrating phlebitis of the superficial veins.

It would be superfluous to present to you tonight a record of all those facts and observations that have been gathered by me from this large amount of material. Nor shall I attempt to go into detail regarding the pathological findings as they are brought to light in the various stages of the disease. I propose rather to call your attention to the conclusions that I regard as significant from the standpoint of pathology and etiology. Thus I may briefly summarize that, in the light of my studies, I believe that I am warranted in thoroughly supporting the proposition that thrombo-angeitis obliterans is an infectious disease in which a specific type of organism is at work; and, although I have not as yet been able to demonstrate either bacteriologically or morphologically the presence of the offending agent, the pathological findings clearly indicate whither future studies should be directed in order that the causative factor may be discovered.

In order that the arguments in favor of such revolutionary views may be followed, permit me to review briefly the conclusions which were drawn from some of my earlier papers.

In May, 1908, I maintained that the pathological pictures could be explained upon the theory that we were dealing with extensive thrombosis in arteries and veins followed by organization and canalization (*International Clinics*, 1909). Most of the larger arteries, and sometimes the veins as well, are obliterated over a large extent of their course.

All stages in the occlusive change may occur in the various vessels in different parts of its course. The closure of the vessels is affected by red obturating thrombi; these become organized, vascularized, and canalized. Recent red thrombosis may involve large portions of arteries or veins and is not secondary to the gangrenous process. It occurs even when no gangrene is present. In short, we are dealing with lesions of considerable extent apparently initiated by the formation of occlusive thrombi, chiefly in arteries, but not confined to these, followed by organization or healing, with an attempt at the production of sufficient collateral circulation.

As to the cause of the thrombosis, my studies failed to reveal anything suggestive. The finding, however, of a peculiar type of histological picture in certain of the veins and arteries, led me to believe, as was stated in my first paper, that the same determining cause which leads to the thrombosis also evokes the changes in the media, adventitia and perivascular connective tissue; and further, that although the mechanical conditions that obtain in the lower extremities and the arteriosclerotic changes, may be factors, some additional agent, be it toxic or otherwise, is at the same time responsible for the production of the periarteritis and the thrombosis.

Unfortunately, we are dealing with the vessels obtained by amputation and it is practically impossible to obtain material in the early or acute stages of the disease. When limbs are amputated, the disease is usually of long duration and the arteries and veins are already in the stage of organization. The presence of the acute and specific picture in two of the cases, however, was so suggestive as to lead to the supposition that here was the lesion in its earliest stage.

It was most gratifying, therefore, to learn that the subcutaneous veins of the upper and lower extremities may also be affected by the same disease. When in the course of my observations the association of migrating phlebitis of the superficial veins with the symptom complex characteristic of the occlusion of the deep vessels was first noted, it appeared to me of no little moment to determine whether the newly discovered phenomenon was not a manifestation of the same malady. Collecting these cases, I was able to report in 1909, eleven patients who, at one time or another, in the course of the disease, had suffered from thrombophlebitis involving the superficial veins of either the upper or lower extremities. The conclusions arrived at that time may be briefly summarized as follows:

1. The disease thrombo-angeitis obliterans is often associated with thrombophlebitis of superficial veins of the arms and legs.

2. Certain peculiar cutaneous nodosities are characteristic manifestations in many of the cases.

3. The disease of the superficial veins may be subsidiary, or it may dominate the clinical picture. Objective signs referable to these vessels should be regarded as extremely suspicious marks of the synchronous development of thrombo-angeitis obliterans. In the presence of migrating phlebitis or cutaneous nodosities we should carefully search for evidence of thrombo-angeitis obliterans, in the form of pulseless vessels, erythromelia, blanching of the leg in the elevated posture, cold and blue toes, pain in the calf of the leg brought on by walking, and other typical phenomena.

4. Migrating thrombophlebitis may give no symptoms, the signs referable to the deep vessels being of most importance.

5. Patients may suffer at one time from the migrating thrombophlebitis, at another from the progress of occlusive change in the deeper vessels.

6. One of the cases suggests the possibility that attacks of

trouble in surface veins may occur simultaneously with similar exacerbations of disease in deep vessels of another limb.

7. The morbid process resulting in the production of cutaneous nodosities and thrombosed superficial veins is not dependent on varicosities, infections, or trophic disorders in the territory which they drain.

8. The vessels of the upper extremity may be affected by the lesion thrombo-angeitis obliterans.

9. Thrombophlebitis in the arm and forearm should arouse our suspicions as regards involvement of the deep vessels of the legs.

10. Further studies should be directed towards solving the relationship between the two thrombotic lesions that we have described. Perhaps excision of nodules and veins early in the disease, exploratory incision for inquiry into the condition of the deep vessels, and bacteriological and serum investigations along the proper lines, will do much to enlighten us in our interpretation of this most puzzling symptom-complex. Although absolute proof is lacking, it seems more than probable that the same determining causative factor is responsible for the lesions of both the superficial and deep vessels.

Since 1909 I have been able to gather data on twelve additional cases in which the superficial veins were involved and have brought the number of excised veins to twenty-five. In these, both the acute and healed stage of the disease were found. From a consideration of the pathological picture the conclusion was reached that a specific characteristic lesion of thrombo-angeitis obliterans may affect the deep as well as the superficial vessels, and that it is from the lesions in the veins that we shall be able to gain access to material for investigation of the causative agent.

In the photomicrographs which I shall now show, and which were kindly prepared for me by Dr. F. S. Mandlebaum and by the Photographic Laboratory of the Cornell Medical School, I shall endeavor to point out the general appearances of the old lesions in the deep vessels, the acute type of lesion in the deep veins and arteries, the lesions as seen in the veins under the skin and how these latter are identical with the lesions of thromboangeitis obliterans, the explanation for the peculiar histological pictures characteristic of the disease, and finally why the findings point so suggestively to the presence of some microbial agent, although none such has as yet been revealed.

Although in this rapid review of a subject which still presents so many mooted points for investigation, we have been unable to give a comprehensive idea of all the data acquired, and although it has been impossible to give all the details which support my contentions, it is hoped that a vigorous plea in favor of the correctness of the following points has been made:

That the so-called disease endarteritis-obliterans is a process in which an acute inflammatory lesion together with occlusive thrombosis of arteries and veins is the characteristic lesion, Second, that from the mechanical point of view and from the symptomatology the thrombotic occlusion is the most important phenomenon.

Third, that the thrombosis is probably preceded and certainly accompanied by an acute inflammatory stage.

Fourth, that the lesion involves deep veins in about 40 per cent. of the cases, the superficial veins of the upper and lower extremity in 20 per cent. of the cases.

Fifth, that recent study on the veins shows that the acute lesions in the superficial vesesls and in the deep vessels are identical.

Sixth, that the histologic changes in the veins point to the existence of an infectious process.

Seventh, that we believe that future studies should be directed towards the discovery of the causative agent in specimens cut out from the superficial veins that are the seat of the acute thrombophlebitis. In this way it may be hoped that the true nature of the disease may soon become revealed.

Discussion:

Dr. MacCallum: There is nothing to add to Dr. Buerger's paper which is perfectly clear. There are two types of disease in these peripheral vessels. In one the lesions begin with calcification of the media followed by alteration in the intima and may result in thrombosis. Such vessels can be seen plainly in the later stages in X-ray photographs with all their branches showing even through the bones. In the case which Dr. Buerger has described, the vessels do not show at all in such photographs.

I am disappointed that Dr. Buerger cannot yet give us the etiological factor which seems upon the point of discovery. It will be interesting to know in connection with the canalization to what degree the vessels are open beyond the thrombus. The canalization may be caused by retraction of the thrombus or by growth of the vasa vasorum into it with anastomoses so as to open the channel through the thrombus and these two processes can be seen side by side, those vessels from the vasa vasorum having distinct muscular walls, while the others, for a time at least, are merely endothelial-lined clefts. I still feel that the part played by the endothelium consists essentially in re-lining spaces and not in the formation of the connective tissue which replaces the thrombus.

Dr. Bishop: My discussion must be a negative one, but the negative side is important. In following arteriosclerosis as my favorite clinical subject, my studies showed that the disease under discussion had nothing to do with the cardio-vascular renal disease that the average physician has so often to deal with. Cardio-vascular renal disease is a localized manifestation of a general disease. I think it is important that the clinician should make the distinction. When he concentrates his mind on the lesion of the arteries that accompany it he feels hopeless from the therapeutic standpoint. It is important to realize that the disease described in this paper and other diseases of the arteries proper exist and to study the relation these diseases bear to the gneral clinical disease known as arteriosclerosis. I am very glad to have heard the paper.

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DR. ALWIN M. PAPPENHEIMER, *President*

THE VALUE OF THE ABDERHALDEN TEST FOR CANCER[1]

ISAAC LEVIN, M.D.

(From the Department of Cancer Research of the Montefiore Home)

An early diagnosis of cancer will probably always present the most important factor in combating the disease. The search for a serum appears to be the most promising line to the majority of the investigators of the subject. In order to be specific for cancer the test must be positive in a high percentage of cancer cases and at the same time must be negative for all other clinical conditions. All attempts to obtain such a test failed up to the present, notwithstanding the vast amount of work done on the subject.

Abderhalden's test for the diagnosis of pregnancy is considered to be specific by a great many investigators. While non-pregnant individuals may give a positive reaction, a negative

Abderhalden test seems to be absolute proof that the individual is not pregnant. In view of this applicability of the pregnancy test Abderhalden himself and several other investigators attempted recently to utilize the method for the elaboration of a diagnostic test for malignant diseases.

In order to form a correct estimation of the results obtained it is necessary to give a brief review of Abderhalden's conceptions of the mechanism of his serum reaction in pregnancy. The following observation served as the basis for the development of the test: When foreign proteid substances like gelatin, casein, gliadin, etc., are introduced directly into the circulation of an animal, there appear in its blood serum proteolytic ferments capable of splitting up the introduced substances and thus render them innocuous to the organism. Abderhalden at first thought that these ferments are formed by the leucocytes. In his most recent publication he brings forward experimental evidence which seems to indicate that these ferments are derived from the cells of the pancreas and the intestinal wall. These cells form and mobilize the same ferments in the course of normal digestion of food. Only in the latter instance the ferments appear free in the digestive tract, while upon introduction of foreign proteids into the circulation the ferments are mobilized directly into the blood serum. These ferments are *not specific* in their action and are capable of splitting up all kinds of proteid material. Abderhalden then took up the question whether similar ferments would be mobilized into the blood serum upon the introduction of proteid substances native to the organism but which under normal conditions do not appear in the blood. Schmorl, Veit, and others have shown that chorionic epithelium enters the circulation during pregnancy. These cells present substances which are not only native to the organism but are produced by the organism itself. At the same time they are not present in the blood serum under normal conditions. It appeared to Abderhalden that these chorionic cells circulating in the blood of pregnant women may mobilize ferments into the blood serum. Investigation showed this supposition to have been correct. There are present in the blood serum of pregnant women ferments which split up the proteid substances of the human placenta and which cannot be found in the blood serum of a non-pregnant individual. These latter ferments in contradistinction to those mobilized by introduction of foreign proteid substances seem to be *specific* and do not split up any other substances but the placental proteids. The free circulation of the chorionic villi in the blood does not occur with sufficient frequency to explain the apparently constant presence of the specific ferments in the serum. Abderhalden thinks that chorionic epithelium may remain *in situ* and send into the circulation proteid material, which may be then split up either by a non-specific ferment derived from the pancreas or by a specific ferment produced by the same chorionic cell and mobilized into the circulation. He further argues that any somatic cell in the course of its metabolism may mobilize into the circulation both its own proteid material and its own specific ferment capable of digesting the former. Again, when a somatic cell dies within the organism it is split up into various proteid substances, which enter the circulation. During this process of disintegration there may be mobilized into the circulation the so called autolytic ferments, which may have a specific action on the proteid material of the disintegrated cell. Thus there may be present at any given time in the blood serum a great variety of ferments both specific and non-specific in their action. It is possible that the sum total of their combined activity may be a proteolytic action specific for a given proteid substance and blood serum? This is the crucial question in the whole problem.

The specific proteolytic action of the ferments present in the blood serum of pregnant individuals on placental tissue seems to be well established though L. Michaelis, and Lagermarck, Oeller and Stephan, and a few others maintain that the same ferments capable of splitting up placental proteids are occasionally found in other diseases, in normal individuals and even in males. Abderhalden states that the test was proven to be correct in at least 1500 cases of pregnancy in Germany alone. In any event a negative test seems to exclude pregnancy completely.

P. Hirsch, Rosenthal and Bieberstein have shown experimentally that there may appear in the blood serum of animals ferments which act specifically on proteid material of certain organs. In view of all this it is possible *a priori* that there may appear under pathological conditions in the blood serum specific ferments acting on the proteid of the diseased tissue. The blood serum of a cancer patient may then be found to contain specific ferments acting on cancer tissue. Such a ferment in order to be fully specific and of value for purposes of diagnosis should not be present in any but cancerous serum.

The results obtained thus far by the different investigators on the subject are very contradictory. Epstein, Gambaroff, and later with improved methods, Guggenheimer, and Abderhalden himself found nearly always positive results with carcinoma serum and negative with serum of non-cancerous individuals. On the other hand, Markus, Fraenkel, Halpern and Oeller and Stephen found a positive reaction only in from 30 per cent. to 75 per cent. of the carcinoma cases, while a certain number of non-carcinomatous sera reacted positively with cancer tissue. The latter investigators came to the conclusion that with the present methods the test cannot be considered specific for carcinoma.

The present investigation was undertaken in order to form an estimate as to the adaptability of the method for clinical purposes and, furthermore, to investigate whether radiotherapy would influence the content of specific ferments in the blood serum of carcinoma patients. The latter phase of the problem has not only a practical but a theoretical interest. It is known that Roentgen rays destroy carcinoma cells. If the ferments of the blood serum which split up carcinoma tissue are derived from dead cells, *i. e.,* are autolytic ferments, then they ought to increase after radiation. On the other hand, if the ferments are mobilized by the carcinoma cells in the course of their life metabolism, then they ought to diminish after treatment, and the Abderhalden test must show it. A few weeks ago while this investigation was in progress there appeared a publication by Oeller and Stephan in which they consider the same subject. They observed the appearance of ferments in the blood serum of patients after radiation, which showed a negative reaction before treatment.

The results of the present investigation were obtained by the aid of the dialysation method. Briefly the test consists in the following: Human carcinoma tissue is boiled in a prescribed manner in distilled water to which acetic acid is added. Small bits of the tissue are placed together with a certain quantity of blood serum in a dialyser of porous parchment paper. The latter is shown in a preliminary test to be permeable for peptone and not permeable for albumen. The dialyser with its contents is put in a glass tube containing sterile distilled water. The water in the tube is covered with toluol to prevent bacterial contamination, the tube is covered with a sterile cotton plug, and the whole placed in an incubator and incubated at 37.5° C. for 16 hours. Then the dialyser is removed from its container, and 10 c.c. of the dialysate free from toluol is mixed with 0.2 c.c. of a 1 per cent. solution of ninhydrin and boiled. Ninhydrin is a substance which gives a colorless solution with water, but when mixed with a solution containing albumen, peptone or amino-acids, gives a violet color on boiling. The more proteid substances there are in the solution the deeper is the color. Since the dialyser is not permeable for albumen a violet color indicates that the blood serum contained ferments which split up the coagulated proteid substances of the boiled carcinoma tissue. Control tests with identical dialysers must show that neither the blood serum alone nor the tissue alone contain any dialysable substances. All the minute details of the test and all the possible chances for error can be found in Abderhalden's most recent publication.

The tests of the present investigation were done under strict guidance of the rules laid down by Abderhalden. The tissue used was adenocarcinoma of the breast and it served for all the cases. Some investigators controlled their results by using also non-carcinomatous tissue. This seems to the writer to be superfluous. The question to be investigated is whether there are present in the blood serum of the patients specific ferments capable of splitting up coagulated proteid matter specific for carcinoma tissue. Blood serum was obtained for the test from 13 cases of carcinoma, 4 cases of sarcoma, 12 cases of pulmonary tuberculosis and from 8 cases suffering from light nervous ailments.

The results of the tests are as follows: Of the 13 carcinoma cases, 12 were positive and 1 negative. The sarcoma cases were all positive. Of the 12 tuberculosis cases, 5 were positive and 7 negative, and of the last series 4 were positive and 4 negative. Subsequent tests taken on carcinoma cases after several X-ray treatments did not show any difference in the ferment contents of the blood serum.

The analysis of the results shows that while the test is most frequently positive in carcinoma it cannot be considered actually specific since the same ferments seem to appear in the blood serum of non-carcinomatous individuals. Abderhalden claims that results negating his conclusions are due usually to faulty technique. But the same faults which increase the number of positive results in non-carcinomatous individuals, would also have shown the number of positive cases in carcinoma to be greater than in reality. Furthermore, the test while consisting of a great many steps is not difficult of execution. The real difficulty lies in the complexity of the phenomena involved in the problem. Carcinoma tissue used for the test consists of cancer cells, connective tissue and blood vessels and consequently must contain different proteid substances some of which may possess characteristics of carcinoma tissue, while others may be analogous to normal tissue. The blood serum on the other hand may contain a variety of specific and non-specific ferments. All these may combine to give a positive ninhydrin reaction. The remedy offered by Oeller and Stephan to take instead of carcinoma tissue emulsions of normal cells homologous to the organ in which the primary carcinoma developed could not serve the purpose. Ferments which may also attack normal organ cells cannot be specific for carcinoma. At best they can show that carcinoma cells are structurally identical with the organ cells in which they grow, and then we may never hope to obtain a specific diagnostic method for carcinoma. In normal pregnancy the conditions are simpler and easier to handle. Nevertheless, the applicability of Abderhalden's test for the diagnosis of pregnancy and the results of the experimental work on animals indicates that action of the specific ferments may dominate the results in a given case. It is imperative therefore that a great deal more of research both clinical as well as experimental should be done before a decision can be reached as to the applicability of the test for the clinic. The proteid substance which the ferments split up must be made to be more clearly specific and the test itself must be finer and not depend on the behavior of the dialyser. All these matters are the subject of further research of the writer. But for the present the method belongs to the research laboratory and not to the clinic.

Discussion:

Dr. Lambert: Can you use in the test tissue from any type of cancer or are the results more satisfactory when the tissue comes from a cancer of the same type as that with which the individual is suffering? Theoretically, it would appear that the best tissue for the reaction would be that from the patient's own tumor.

Dr. Levin: We can't use cancer tissue from the individual because we do not always possess it. As far as the use of homologous tissue is concerned, if the test is to be of any use at all we should be able to find out whether there is cancer or not. I used throughout the same tissue, adenocarcinoma of the breast; it sufficed for all of the work. Others have used tissue from two or three organs. One investigator claimed, as is stated in my paper, that he obtained a better result with cells of the normal organ liver for instance in which the tissue grew than with carcinoma cells of another organ. Such results, if correct, would appear to me to completely invalidate the test.

Dr. Lambert: Judging from the morphological characteristics of tumors, it would appear that the cells of the various types of cancer may differ from one another more widely chemically than from the organs in which the tumors arise. For example, the cells of an adeno-carcinoma of the liver are probably more closely related in their chemical structure to normal liver cells than they are to the cells of a squamous cells carcinoma of the skin.

Dr. Strong: I undertook the Abderhalden test with a view of using it for the diagnosis of cancer and my results were very unsatisfactory. I went back to the pregnancy test and in 150 tests found it very unreliable. I could not draw definite conclusions and discarded the whole test. The criticism has been made that the technique was wrong. Ultimately that criticism must fall to the ground for so many workers have had the same results. Abderhalden has recently said that only those who were able to make the distinction between pregnancy and non-pregnancy could do the test. If one got a reaction in a non-pregnant case he could not do the test. Later he published such a result himself.

Dr. Levin: In justice to Abderhalden, I must say that the easiest way to get the technique of the work is to try it on pregnancy before undertaking other work. As for the idea that whoever gets results negating Abderhalden's work does not master the technique,—the technique is complicated, but not more so than a number of other tests.

THE EFFECT OF PROPHYLACTIC TUMOR VACCINES IN RATS

RICHARD WEIL, M.D.

The fact is probably familiar to all, and therefore hardly needs emphasis, that it is possible to inoculate experimental animals with tumor tissue, or even normal tissue, and so to put them in a condition in which they are refractory to subsequent tumor grafts. In an article published this year I stated that it was possible by making a thin suspension of tumor cells and injecting this in small amounts into the jugular vein to produce multiple pulmonary growths. As these pulmonary tumors have never been shown I have brought specimens this evening. When these suspensions were injected in the same amounts in the skin, they almost invariably failed to produce grafts; in animals so treated, the tumor would not grow on subsequent inoculation.

In a series of experiments I combined the subcutaneous and intravenous routes, vaccinating intravenously and subcutaneously at the same time. In the first set 7 control animals were used; they were injected intravenously and after 24 days all had tumors of the lungs; 6 received, in addition, subcutaneous injections of the same suspension. In these latter, which were killed in from 21 to 30 days after injection, there was no tumor growth. In a second series the same results were obtained. A specimen of a control rat of the series is shown with the lungs riddled with tumors. The animals which were injected both intravenously and subcutaneously on the same day with the same suspension presented no pulmonary tumors.

It seems fair to conclude that in case of simultaneous injections by the subcutaneous and intravenous routes, if the animal is refractory to the subcutaneous and susceptible to the intravenous, that the immunity developed by the former route prevents the "take" of the pulmonary graft. In 6 animals the subcutaneous vaccination was done six days after the intravenous injection. They were killed after 28 days and the lungs of 5 were negative and 1 positive. In a third set the subcutaneous injection was done ten days after the intravenous injection. The animals of this series were killed after 26 days and presented pulmonary tumors.

These experiments show that the animals can be protected by subcutaneous injections against a simultaneous or a preceding intravenous injection of tumor tissue. The protection was pronounced up to six days; after ten days the protection was very slight.

Discussion:

Dr. Wood: What anatomical condition was found in the lungs of animals which had had an intravenous injection six days previously? If the cancer emboli in the lungs after six to ten days had already formed firm tissue attachments, Dr. Weil's experiments would show the possibility of influencing tumors after they had begun to grow. On the other hand, if the emboli were still in the condition of those described by M. B. Schmidt, covered with fibrin and not actively growing, it would show that the tumor cells merely had been influenced and not the tumors. It would be a very important thing if actively growing tumors could be influenced in this way.

Dr. Weil: I can not answer Dr. Wood's question. I sectioned a number of lungs but on account of the small size of the grafts, which consisted of six to eight cells only, I found it impossible to demonstrate them in sections.

THE INFUENCE OF DIET ON TRANSPLANTABLE SARCOMA IN RATS

S. P. BEEBE, M.D.

The first paper published by us upon the question of the effect of diet in cancer had to do with the results obtained when carbohydrate was entirely eliminated from the diet. We found that if carbohydrate was entirely eliminated there was a smaller percentage of takes, the tumor grew much more slowly, and a larger percentage showed complete regression as compared with control animals on full diet.

In one experiment 26 animals were placed on a special diet, and 26 controls on a full diet. They were all inoculated with the same tumor on the same day. Every animal inoculated showed a "take." Of those animals on the special diet containing no carbohydrate, 2 died from the tumor, 4 died from accident or unknown causes, and 20 showed complete regression of the tumor. Of the 26 animals on the control diet containing carbohydrate, 24 died from the tumor and 2 recovered.

In order to obtain such effects, however, it was found necessary to prepare the animals by feeding them on a special diet for some time (two to six weeks) before inoculating them with the tumor. If a special diet was begun on the day of inoculation no difference could be observed between the two groups of animals.

The most recent experiments have had to do with other modifications of diet. It will be recalled that Mendel and Osborne have shown that the normal growth of a rat is markedly affected by the character of fat in the food. If a healthy rat is placed on a liberal diet containing fat in the form of lard he grows well for a time but finally reaches a stage where growth is very slow, and finally a loss of weight occurs even though he has an abundance of food. If, at this time butter is substituted for lard, growth begins again at once and continues in normal fashion. If the animal is fed on butter from the beginning there is no evidence of a cessation of growth.

We have made experiments to determine whether such an effect as was observed in normal animals would be produced in tumors. Our experiments have shown that butter is a very effective stimulant to the growth of the Buffalo rat sarcoma, the tumor used in our experiments. The tumors grew much more rapidly in the animals receiving a butter diet. A much larger percentage of the animals was killed by the tumor when the animals were fed on butter.

In these experiments it has not been necessary to have a previous preparation of the animal in order to get the results indicated.. The tumor growth in the animal fed on butter has been the most rapid and the tumor has reached a much larger size than in any animals that I have seen before. In some instances at the time of death of the animal the tumor mass weighed two and a half times as much as the rest of the animal. In one instance the weight of the tumor dissected out at the time of death was 130 gm., while the weight of the body of the animal after removal of the tumor was only 51 gm. From this experiment it appears that the diet favorable to the growth of animal tissue is also favorable to the growth of tumor tissue. Is it not difficult to reconcile such observations with the theory that these tumors have an infectious origin?

We have made some experiments to determine the effect of cholesterol on tumor growth but we are not yet ready to publish any conclusions.

It will be recalled that Mendel and Osborne have found that certain proteins which were incomplete chemically permitted maintenance but prevented growth. We have made some experiments with a diet containing protein of this type and have found that it is possible to maintain an animal in a fairly satisfactory condition of nutrition and yet prevent growth of the tumor. It has been possible to do this likewise in animals which have not been prepared for the implantation of the tumor by a previous period on a special diet. A considerable number of factors influenced the results which may be obtained in experiments of this character. Experiments have been made with other types of protein food but we are not yet ready to report results at this time. Enough has been determined, however, to indicate that the character of the diet influences the growth of the implanted tumor to a remarkable degree.

These statements are based on experiments with the Buffalo rat sarcoma. We are not yet prepared to say what results may be obtained with other types of tumors.

THE INFLUENCE OF UNDERFEEDING ON SPONTANEOUS MOUSE TUMORS

PEYTON ROUS, M.D.

(From the Laboratories of the Rockefeller Institute for Medical Research)

Several workers have shown that underfeeding or the feeding of special diets will render mice relatively insusceptible as hosts for transplanted tumors. In general the results have been obtained by beginning the treatment at the time of inoculation of the animals or several weeks previously. The question whether large tumors, recurrences after operation, or metastases can be influenced by diet has not been adequately studied; and the influence of diet upon spontaneous growths has never been investigated experimentally.

The experiments now to be reported have been carried out with the help of Dr. Linda Lange. They are a continuance of work begun four years ago and already briefly reported upon.[1] At that time it was found that whereas in animals underfed on a standard diet grafts of transplantable tumors "took" with relative difficulty and grew slowly (Moreschi), if the underfeeding was begun after the tumor had reached a considerable size its effect on the neoplasm was much slighter. Indeed the Flexner-

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[1] *Proc. Soc. for Exper. Biol. and Med.*, 1911, VIII, 128.

Jobling adenocarcinoma grew with the same rapidity in rats rapidly losing weight on low diet as in controls fattening on the same kind of food. The Jensen sarcoma, a growth of relatively unstable equilibrium, and one which frequently retrogresses after attaining a large size, grew slowly and retrogressed more frequently in underfed hosts. Our recent experience has made it apparent that the behavior of these two growths may be taken as type instances.

In resuming the work, tumors of the mouse as well as of the rat have been employed. For rats the same ration as previously has been used,—a finely ground cake of oatmeal, rye-flour, corn-meal, milk and sugar with measured quantities of fresh milk to moisten it. The mice were underfed on a diet lacking in certain constituents necessary to growth, namely on Sweet's modification of one of Mendel's foods.

With the idea of studying the influence of underfeeding on the development of metastases two mouse tumors which frequently metastasize were procured. But the underfeeding so markedly delayed the enlargement of primary growths of both sorts that to test its effect on secondary tumors seemed unnecessary. The influence of underfeeding on recurrences and implantations of the Flexner-Jobling tumor, following operation, was next taken up. Rats carrying large growths of this strain were underfed and when they had lost much weight were operated upon and the tumor removed save for a small bit left with its vascular connections undisturbed. Two subcutaneous implants of the animal's own tumor were made at a distance from the wound. These may be taken as representing in some sort disseminations at the time of operation. It was found, just as in the experiments of four years previous, that underfeeding carried to the point of emaciation had no effect on the primary growth. The result as regards recurrences and growth of the implants varied from series to series. In one series recurrences occurred more promptly and the implants grew more rapidly in the underfed rats than in the controls. In another set opposite results obtained. In a third series the underfeeding had no obvious influence. Altogether it was plain that many factors besides mere differences in nutrition entered into and served to confuse the results. One such factor undoubtedly is the resistance engendered in the host by a transplanted tumor acting as strange tissue. The conclusion seemed unavoidable that findings with transplanted growths could not be used for generalization as regards the influence of diet upon spontaneous tumors.

To obtain spontaneous growths of the mouse proved relatively easy; and thus far about one hundred have been used for the work. All have been growths of the female mamma and all adenocarcinomata except three, these latter being carcinoma sarcomatodes. The mice were divided into two lots, one full fed on a mixed diet, the other underfed on Sweet's modification of Mendel's food. All were operated upon by a uniform technic and the growths removed save for a small bit. Two subcutaneous implants of the animal's own tumor were made. Following operation the animal was kept under observation for thirty-five days, a period within which, as Haaland has shown, spontaneous growths ordinarily recur in full-fed mice.

The operative mortality was high and the results in a number of animals were ruled out because of complications. Among the remaining mice 83 per cent. of 24 fed on full diet developed a recurrence within 35 days, as compared with 41 per cent. of 22 underfed mice. The grafts grew in 41 per cent. of the underfed and in 68 per cent. of the controls.

It is possible, then, by underfeeding on a suitable diet, to delay recurrence and the growth of implants of a proportion of the spontaneous tumors of the mouse. The period of thirty-five days in the mouse's life corresponds to about two years in that of the human being. Unfortunately even in mice in which underfeeding gave its best results no enduring resistance was acquired. When the animals were placed on ordinary diet and regained weight a recurrence appeared and the grafts grew, usually very promptly.

The loss of weight entailed by the underfeeding was considerable. This fact seems to have influenced the results more than the character of the diet, to judge from the results in a recent series of mice in which the special food was given in quantity and the weight altered but slowly. In these animals the findings were little different from those in the controls on full diet.

It was repeatedly noted that the healing of the wounds of underfed mice took place with great slowness. Special experiments show that the proliferative activity of the connective tissue of such animals is much decreased. This may furnish some explanation of the results with the tumors. For those growths markedly influenced by the underfeeding were in general relatively benign ones, such as do not invade but are dependent for the most part on a new, supporting and vascularizing stroma elaborated by the host. Any factor retarding the formation of this stroma would doubtless retard indirectly the tumor growth.

Discussion:

Dr. Levin: The growth of a tumor is a complicated phenomenon and differences in food alone cannot explain all the variations. Dr. Rous's charts which deal with operative removal of spontaneous tumors interest me particularly. I have met the same condition, when I removed a tumor transplanted subcutaneously and then inoculated a piece of it into an organ (liver or spleen) of the same animal. There was no change in the diet. Whenever the subcutaneous tumor recurred after the operative removal the graft in the organ grew. When there was no recurrence of the subcutaneous tumor, there was no take in the organ. Apparently in the latter animals there has developed a general immunity which had no relationship to the conditions of feeding. Is Dr. Rous certain that all of his tumors were spontaneous?

Dr. Ewing: It would seem natural that the exaggerated growth of neoplasms should be partly inhibited by withdrawal of food. Experimental proof of this fact has not been before furnished. Dr. Rous concludes that this fact can have no bearing in human tumors, but I am told that there is a successful sanitarium in this state where cancer is treated with remarkable results, chiefly by starvation. The influence of low diet is not an entirely new observation, but the demonstration that tumors can be affected by withdrawal of certain substances from the diet which are especially favorable for tumor growths, without starving that animal, introduces a new field into modern cancer research. It would be interesting to know if the Flexner-Jobling tumor which refuses to respond to low diet, can be influenced by variations in carbohyrates, lard, butter or cholesterol.

Dr. Rous: It has often been observed, of course, as Dr. Ewing says, that the tumors of human beings are influenced by sickness or emaciation of the host. But this matter has not been studied with the spontaneous growths of animals. That is the reason for the work just presented. It has seemed of importance to learn what proportion of spontaneous tumors can be influenced by underfeeding, and whether in the case of neoplasms held in check by underfeeding the host develops some resistance. We have asked ourselves also how rigorous underfeeding must be in order to affect recurrences and implants, and furthermore whether spontaneous tumors as well as transplanted ones can be controlled by a food that prevents growth.

Dr. Levin has suggested that our spontaneous mouse tumors were in reality transplanted ones sold by dishonest dealers. This possibility can be ruled out. The growths had all the peculiarities of spontaneous tumors, many of them being hemorrhagic adenocarcinomata such as are rarely transplantable. Furthermore they were, histologically, of great variety. This would not have been the case had they resulted from transplantation. Finally, they were practically all furnished to us by two breeders of mice of whose reliability there is no doubt.

THE ACTION OF RADIUM ON TUMORS OF MICE

F. C. WOOD

A decision as to the final effect of radium on cancer is not possible at the present time. Radium may influence some of the benign types of epithelioma which occur about the face and scalp and some but not all of the myeloid bone tumors, but it is certain that it does not as at present applied act favorably on many of the tumors of the mucous membranes and internal organs. The many difficulties which attend the scientific study of the action of radium on human beings led the writer to begin a series of experiments on the action of radium on spontaneous carcinomata of mice. Previous studies have all been made on transplanted tumors of mice and rats, but even if radium should act favorably on these tumors there would be no proof that it would act favorably on spontaneous tumors.

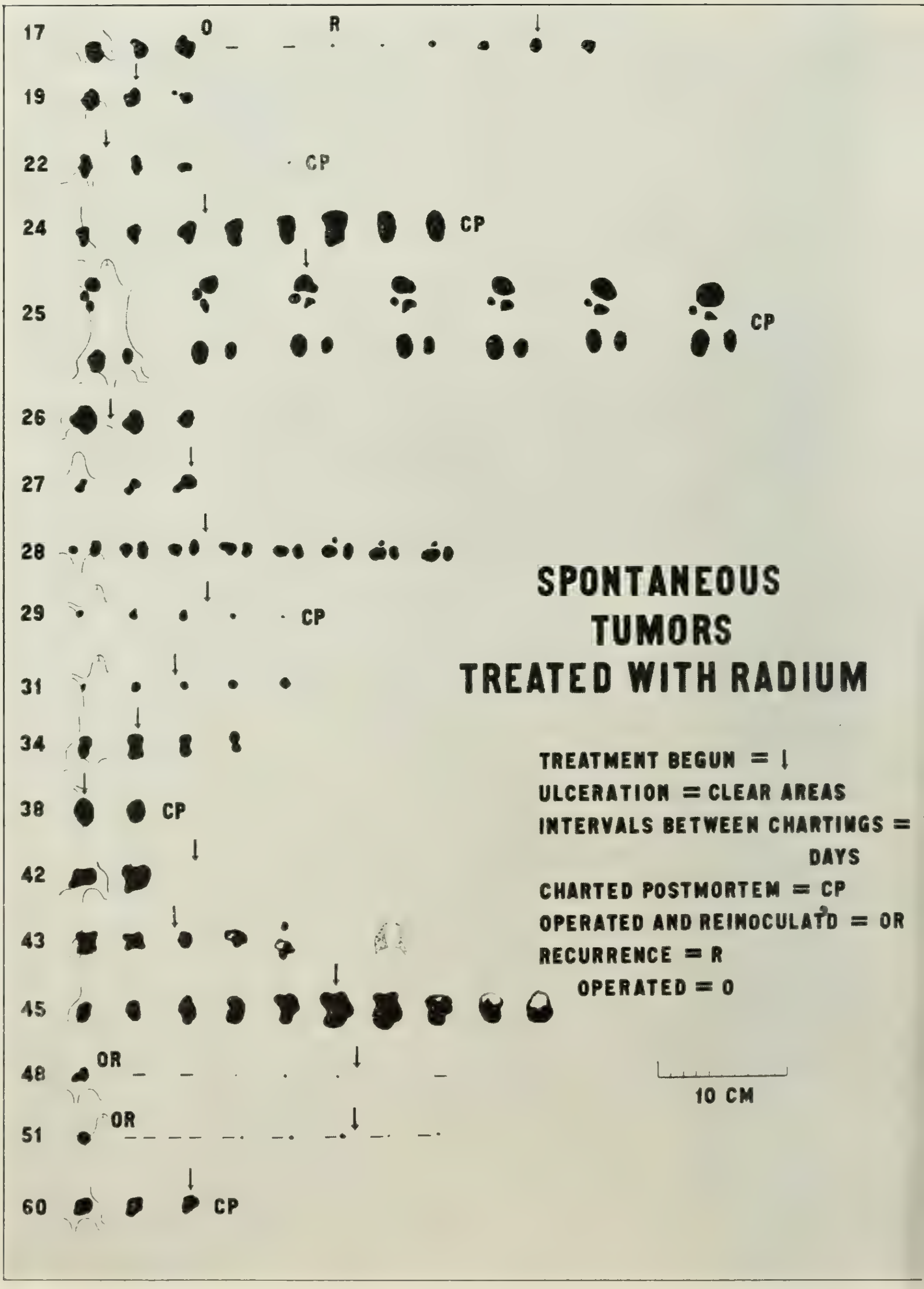
Apolant, Bashford, and Maud Menten have all reported certain rather doubtful effects on transplanted mouse tumors. Wassermann and Krönig and Gauss say that no effect can be produced on transplanted mouse tumors by the use of large quantities of radium bromide, varying from 15 to 100 milligrams. Wassermann quoted Lazarus as having had some favorable results. Krönig and Wassermann conclude, therefore, that a mouse carcinoma, even the transplanted growth, possesses a special resistance to radiation, much greater than is possessed by human carcinoma.

Any one who has attempted to cure a glandular carcinoma of the human being with radium will know that that growth also possesses a special resistance to radiation, which is a great deal more effective than the resistance which the mouse tumor possesses. Of course, every one knows that secondary nodules of carcinoma, if they are subcutaneous, can often be cured by radium; but the primary tumor, if of any size, does not yield. It is, therefore, almost certain that if radium will not cure carcinoma of the breast of the mouse, it will not be likely to cure carcinoma of the same organ in the human being.

My own experiments show a marked influence exerted by radium on primary tumors. In no case, however, was a cure effected. A large part of the immediate effect of radium is not, in my opinion, upon the cancer cell itself, but is upon the blood vessels, causing hemorrhage and subsequent necrosis, and only when repeated exposures to large quantities of radium are made is the growth of the cells themselves interfered with. Even so, my own studies, which are as yet incomplete, do not point toward the possibility of ultimate cure.

The radium is used in glass tubes, in flat applicators, or inclosed in metal screens of greater or less thickness. The use of emanation has also been tried by Duane of the Harvard University Cancer Commission, and by Joly. The London Cancer Institute has also used emanations. The advantage of the application of the emanation lies in the concentration of energy which is possible, for the entire strength of a large quantity of radium can be concentrated in a small glass tube of less than 1 mm. cubic capacity. Such tubes may be placed directly in a tumor, and will give off twenty-five to thirty per cent. more energy than the corresponding tubes of radium, for the screening necessary with the radium can be dispensed with when the emanation is used.

The chart (fig. 1) shows the results of my experiments, the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SPONTANEOUS
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TREATED WITH RADIUM
TREATMENT BEGUN = ↓
ULCERATION = CLEAR AREAS
INTERVALS BETWEEN CHARTINGS =
DAYS
CHARTED POSTMORTEM = CP
OPERATED AND REINOCULATD = OR
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OPERATED = O
10 CM

FIG. 1.

system used being that devised by Gaylord and made familiar by Bashford. None of the animals were cured; but some of the cases are of sufficient interest to warrant a detailed discussion.

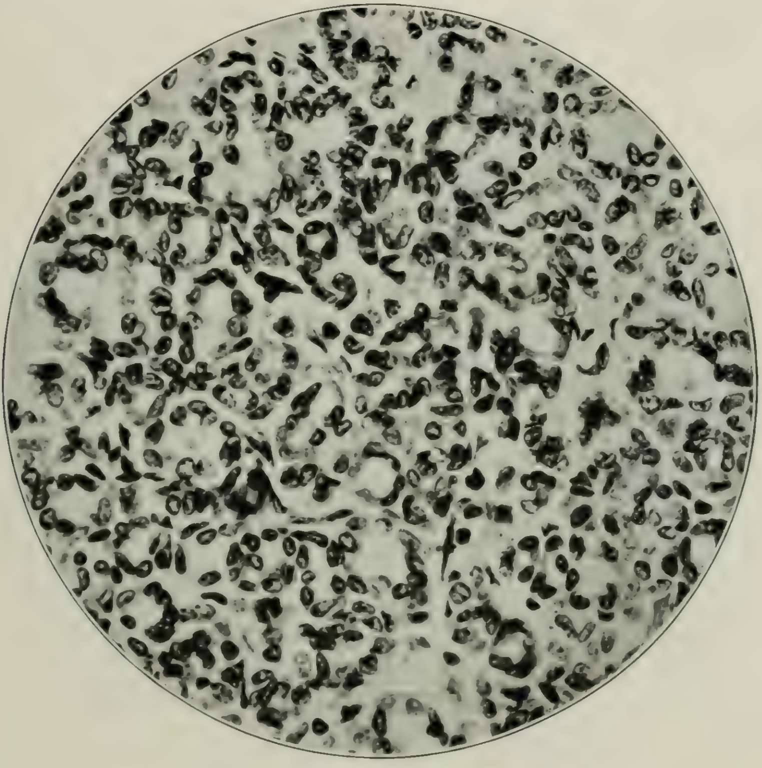

FIG. 2. Adenocarcinoma of breast of Mouse. × 500.

MOUSE 17/0. This animal had six tumors, all of which were excised and examined microscopically (fig. 2). A recurrence of one tumor was noted shortly after, and when this had reached a size of 8 × 10 mm. it was treated with 83 mg. of radium element, corresponding to 140 mg. of radium bromide, inclosed in a platinum tube 2 mm. in thickness and five thicknesses of hardened filter paper (S. & S. No. 575). This was laid in close contact with the tumor for one hour and thirty-five minutes;

and on the two days following for one hour each. Two days later the tumor showed distinct evidences of growth, and the same amount of radium unscreened was applied for one hour and ten minutes. Five days later the animal was found to be dying

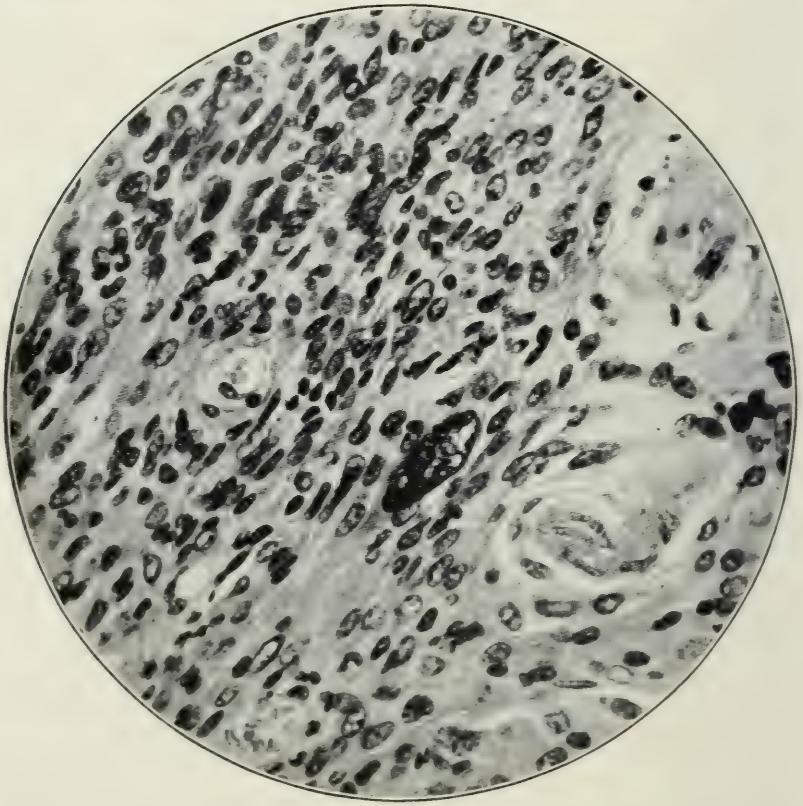

FIG. 3. Sarcoma-like growth following radium treatment of recurrence of tumor shown in Fig. 2. × 500.

and was killed. Examination showed the tumor as a flat oval mass adherent to the skin of the thorax. There was an inflammatory hemorrhagic area in the left axilla near the tumor.

Microscopical examination showed this tumor, which when first excised was an adenocarcinoma, to be altered into a sarcoma-like growth (fig. 3), no recognizable epithelial cells being present. Interpretation of the condition is difficult. We may assume: (1) that the epithelial cells were so modified that they assumed a connective tissue shape at the site of the tumor; (2) that the radium destroyed the epithelial cells and induced a sarcoma by its stimulating power (Clunet produced a sarcoma after x-ray burns in a white rat); (3) that the tumor stroma had recurred as a sarcoma as has been noted by Bashford and Ehrlich: or (4) that it was a granuloma.

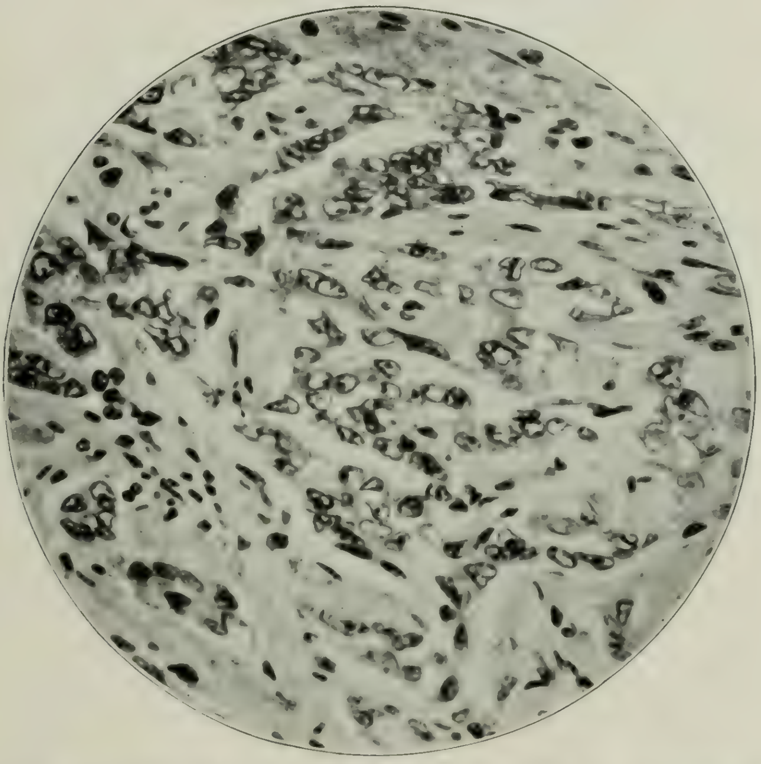

FIG. 4. Shrinkage and syncytium formation as the result of radium applications. × 500.

MOUSE 19 O. This mouse had a tumor measuring 16 × 13.5 mm., and involving the left side of the abdomen and rectum so that it was inoperable. Seventeen mg. of radium element, equal to 30 mg. of radium bromide, inclosed in a glass tube, were attached to the tumor for three hours. A small fragment of the tumor was excised for control and showed that the growth was an adenocarcinoma. Ten days later the animal died. The tumor had shrunk a good deal; it was cystic and filled with chocolate colored fluid. At the portion of the circumference furthest from the radium, normal active tumor tissue was found.

Mouse 22/0. This animal had a tumor, measuring 8 × 11 × 19 mm., occupying the region of the rectum and vulva and the right side of the abdomen. It was treated with 140 mg. of radium bromide, covered with 1 mm. of black rubber, for five times during a period of six days, the total amounting to five hours. Eleven days later the animal died. The tumor, as the chart shows, was only a small nodule, 2 mm. in diameter. Portions of the tumor showed typical radium alterations with shrinkage of the epithelial cells and a tendency to form syncytium with large nuclei (fig. 4). Other areas showed adenocarcinoma with mitoses.

Mouse 38/0. This animal had a tumor in the right axilla, measuring 21 × 15 mm. A glass tube, 4 mm. in diameter, and containing 16 millicuries of emanation, equal to 16 mg. of radium element, was inserted in the tumor under aseptic precautions. The animal remained in good health for eight days, and was then found dead. On removal of the tumor there was no evidence of shrinkage, and no apparent lesions in the viscera.

Microscopical examination showed the tumor to be of the sebaceous type. The central portion showed necrosis. At the periphery there were a few alveoli with cells showing fairly normal structures (fig. 5). While it is impossible to make a definite statement, yet it seems probable that this tumor would have recurred.

Much more striking results were occasionally noted in treating animals whose own primary tumors had been removed and inoculated in another portion of the body. These secondary inoculations always take, and the tumors correspond to metastatic nodules in the human being, so that one would expect a certain improvement under treatment.

MOUSE 51/0. This mouse had a tumor which was removed and inoculated. The implantation was then treated for three hours with 140 mg. of radium bromide unscreened. The tumor shrank a good deal, but it was difficult to determine its exact size owing to the inflammatory tissue surrounding it.

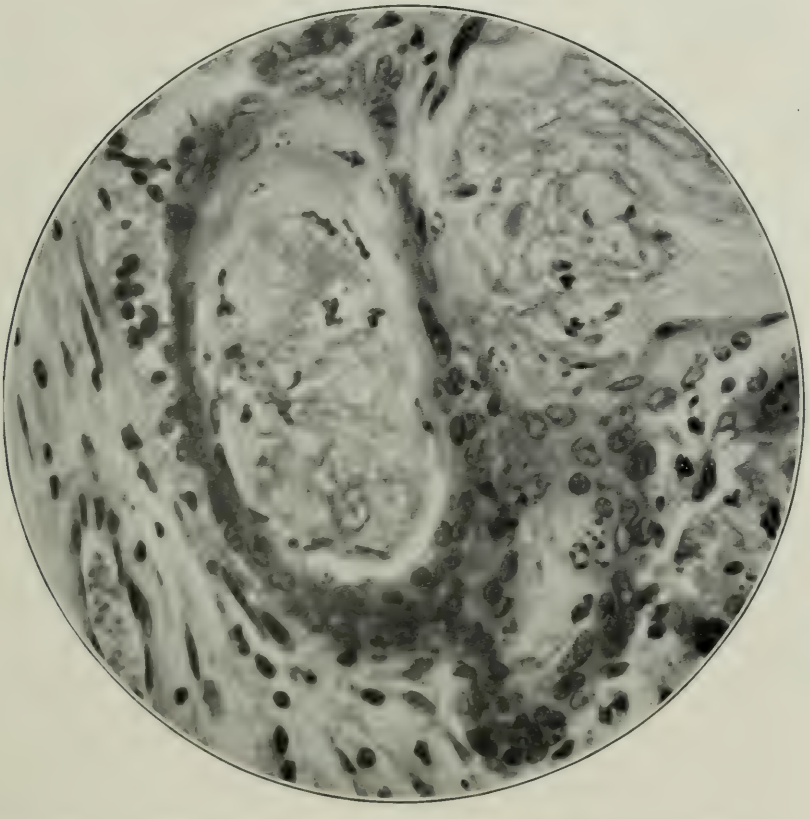

FIG. 5. Showing result of treatment with 16 millicuries of radium emanation on sebaceous carcinoma of the mouse. Well-preserved peripheral portion. × 500.

On microscopical examination, twenty-seven days after the exposure, perfectly normal, actively growing carcinoma was found at the site of the inoculation.

CONCLUSIONS

The action of large quantities of radium on spontaneous tumors of mice is to destroy a considerable portion of the tumor, but in the series so far studied there has been no evidence of total destruction of the tumor mass.

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Dr. Alwin M. Pappenheimer, *President*

FOUR CASES OF CHONDRODYSTROPHIA FŒTALIS

W. G. MacCALLUM, M.D.

Of the four cases presented one was an adult, a woman aged 75, the other three infants.

The woman was 3 ft. 9 in. in height with a huge head, 58 cm. in circumference, and extremely short arms and legs. The pelvis was deformed by the almost horizontal position of the sacrum so that its cavity from the sacral promontory to the symphysis pubis measured 6 cm. The femur measured 22 cm. in length. Ossification was, of course, complete in all bones so that nothing of the nature of the process leading to the deformity could be learned. Details of the autopsy will be published elsewhere, but it may be said here that all of the organs of internal secretion were found to be normal excepting that the pineal gland was very small, a condition probably to be explained as a senile change.

The infants presented the typical deformity so completely described in the papers of many authors. The retraction of the nose, which was only moderate, could not be explained in these cases on the premature synostosis of the sphenoid and occipital bones, for in each case the two portions of the sphenoid and the basilar portion of the occipital were separated by wide cartilages.

In two of them the changes in the developing bone were identical; in the third a different condition was found.

In the first two the periosteal bone formation was vigorous and the periosteal sheath of the diaphysis projected beyond the actual shaft and turned inward into the cartilage. The cartilage at the extremities was present in great bulk but had, as it were, overflowed and slipped down so as to cover the end of the shaft like a cap, a large part of it lying along the shaft outside the periosteum and thus out of the range of the advancing marrow spaces with their blood vessels and osteoblasts. Over the actual end of the bone and within the range of activity of these marrow spaces there remained only a thin layer. This cartilage had lost much of its matrix and consisted of a network of cartilage cells separated by spaces in which fibrils could be seen. In places it had the appearance of a vascular fibro-cartilage.

In the line of ossification bone formation was proceeding but the presentation of cartilage cells was taking place rather irregularly and not always in parallel columns. Premature calcification of the cartilage was prominent in places.

Here the fault seems to lie essentially in the cartilage which fails to maintain the proper order of the cells and to present them and the matrix promptly and in abundance for ossification. Instead the matrix is abnormal in consistence and much of the cartilage is permanently displaced from the range of the process of ossification. In the third case the cartilage was more normal in appearance but again relatively very bulky as compared with the diminutive shaft which in the femur was less than 1 cm. long.

The obstruction to ossification seemed to lie in the fact that the cartilage cells in face of the line of ossification formed no columns at all but merely lay transversely to the shaft of the bone and ended in a sharp line nowhere invaded by marrow spaces. What bone existed had apparently been formed some time before, after which the line of ossification had lapsed into complete inactivity.

In one of the infants the organs of internal secretion were studied with the exception of the hypophysis and pineal gland and were perfectly normal. The other two were old museum specimens in which these organs had disintegrated.

CASE FROM WHICH A SPOROTHRIX WAS ISOLATED

ELIZABETH FINCH, M.D.[1]

(From the Research Laboratory of the N. Y. Skin and Cancer Hospital)

This case can simply be spoken of as a case from which a sporothrix was isolated, for this organism was not proven to be the only etiological factor. The patient had received a year and a half of specific treatment when the bacteriological examinations recorded were made.

The patient, Mr. J. K., an Austrian, 21 years of age, single, tailor by occupation, was admitted to the skin wards of The New York Skin and Cancer Hospital, June 17, 1913, on service of Dr. H. H. Whitehouse, through whose courtesy the case is presented.

Family history: Mother died at the age of 43, presumably of tuberculosis. There was nothing else of importance elicited.

Previous history: An eruption on the face three years before, which lasted three weeks and healed under local treatment. Venereal infections were denied.

Onset of *present illness* occurred in the fall of 1911 with intense headache, pain in the temporal region of four weeks duration. Cessation of pain under internal medication was followed by the appearance of a small papule behind the right ear. This enlarged with abscess formation, and healed, but a much larger

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[1] Bacteriologist to the Hospital and Research Fellow.

"abscess" appeared in the lumbar region. Two Wassermann tests were both negative. The patient received one intravenous injection of "606" and one hypodermic treatment in the buttocks. One week following the latter, there occurred a general eruption on the back of large "pustular ulcers which took over six months to heal."

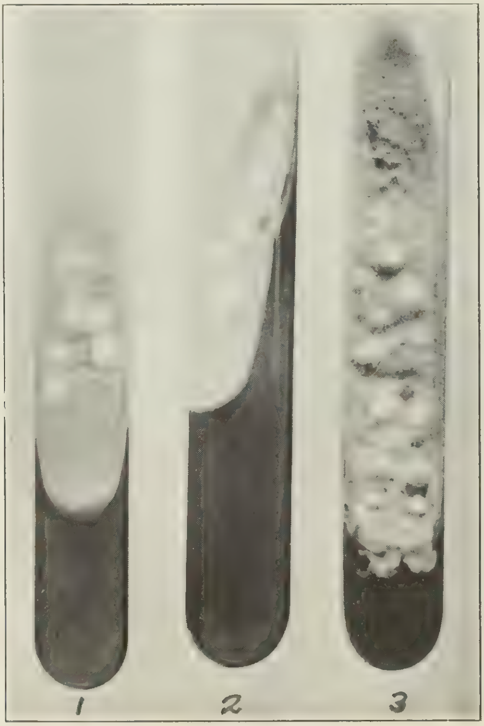

FIG. 1. Culture of sporothrix. 1. One week's growth on glycerin agar showing mycelium with beginning hyphæ formation at top of tube. 2. Two weeks' growth showing surface entirely covered by hyphæ. 3. Older growth showing chocolate-colored pigment.

On admission to the hospital, Wassermann reaction was negative. Back was "covered with a pustular eruption" and depressed pitted scars "slightly irregular in outline," from two to five cm. in diameter. Large unhealed ulcer with undermined edges was present on the face in front of the left ear. Dec. 5, 1913, six months after admission a section was taken for pathological diagnosis from the margin of this same ulcer. The eruption on the back had healed following five intravenous injections of neosalvarsan, and active mercury and potassium iodide treatment, the latter up to forty-five, and for a short period of time, sixty grains of potassium iodide a day. Microscopical examination of the section from the ulcer showed only "acute inflammatory changes." The stain for tubercle bacilli was negative.

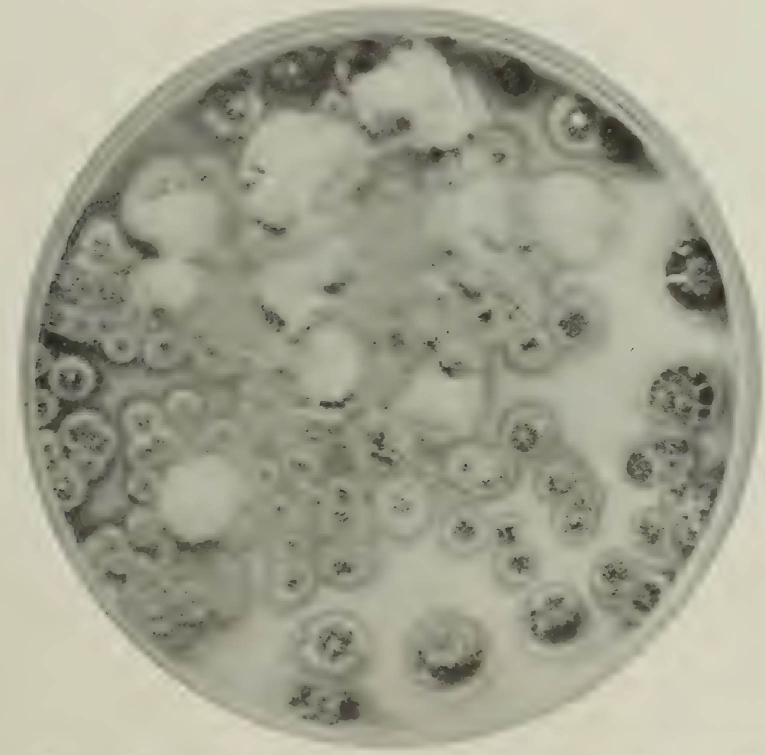

FIG. 2. Three weeks' growth of sporothrix on glycerin agar plate showing beginning pigmentation of culture.

The clinical picture at this time, aside from the scars on the back of the patient, was suggestive of Hodgkin's disease. There was enlargement of the cervical, axillary and inguinal glands on both sides, but more particularly those on the left side of the body. One in the neck over the center of the left sterno-cleido-mastoid, 8 cm. in diameter, showed softening and beginning skin involvement. Slight grade of anæmia was present as shown by blood examinations.

On December 30, 1913, two glands were removed from the left axilla, one, 4 × 3 × 3 cm., was kept sterile and taken to the Loomis Laboratory for culture. The softened gland in the cervical region was punctured aseptically and the contents, ½ c.c. of serosanguinous fluid, removed and cultured. Stained smears of this fluid showed Gram-negative, non acid-fast, slender bacilli. No other organism was observed.

The results of gland culture were as follows: On the second day a bacillus same as the above was found growing on one glycerin blood agar tube; on the seventh day a small round colony with slightly depressed corrugated border and fine aerial hyphæ was found growing on a different blood tube; on the ninth day two similar colonies were present on one glycerin agar tube. Later a colony developed on one of the Loeffler tubes and one of the egg medium tubes. All other tubes remained sterile. Examination of the growth in glycerin and sodium hydroxide showed septate fine mycelial threads with some branching and small round and oval spores. Stained with Loeffler's methylene blue, the mycelium showed two types, that with and that without fine granules in the center protoplasm. The spores took an even intense blue stain or showed a lighter area at one end or both and were found singly or in small groups, and finally in typical arrangement, placed laterally, around mycelial threads. They varied from about 2 to 5 μ in diameter. A few ovoid, cigar-shaped bodies were present in the early transplants.

Paraffin sections of the second gland that was excised from the axilla showed a diffuse growth of endothelial cells with some increase in the fibrous tissue, large numbers of eosinophil cells in areas associated with chronic inflammatory changes, and an occasional young giant cell. Spores of the ovoid and oval type in infrequent numbers were found in the sections.

Growth of the original colonies was slow; the hyphæ and the mycelium of the colonies on the blood tube developed a chocolate-colored pigment. Transplants on glycerin agar did not develop any color during the early stages of cultivation. Now, ten months after isolation the pigment develops as early as the second week on glycerin agar as well as on other media.

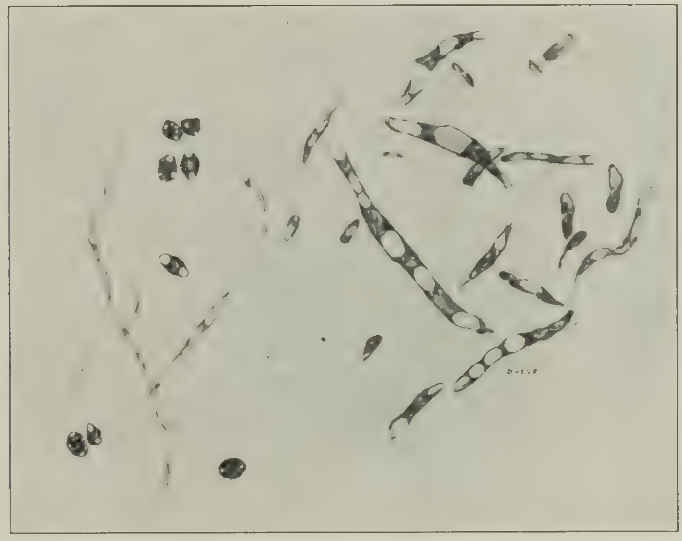

FIG. 3. Sporothrix. Types of mycelium. Several spores are shown on the left.

Subsequent history of patient: Marked reaction followed aspiration of gland in the neck; temperature, prostration, swelling of entire left side of face, with gradual subsidence. As the sporothrix had been isolated while the patient was taking potassium iodide in his mixed treatment, this drug was used in increasing doses over a period of four months, until he received over one hundred and ninety grains a day.

Jan. 17, 1914.—Blood culture negative.

Feb. 9, 1914.—Agglutination reaction, using the spores of the organism and the patient's serum, was negative.

Feb. 14, 1914.—Rupture of gland in the neck, also discharge from the left axilla. Irregular fever, and much pain present in left side of face, eye, ear and mastoid region.

April 28, 1914.—Complement fixation reaction, using the spores as antigen, and immune rabbit serum for control to the patient's serum, was negative.

May 11, 1914.—Potassium iodide was discontinued and treatment thereafter was symptomatic.

June 1, 1914.—Large abscess in left buttocks was opened. Improvement in the ulcer on the face followed the development of this abscess.

July 10, 1914.—Abscess of the right arm near the elbow was opened. Unfortunately, cultures from these two abscesses were not obtained. There was progressive exhaustion of the patient and mental depression.

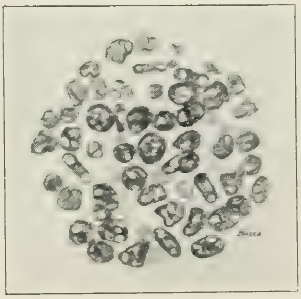

FIG. 4. Types of spores found entering into the formation of the hyphæ of the sporothrix. Taken from a paraffin section of the organism grown on glycerin agar and stained with methylene blue.

July 30, 1914.—Transferred to another hospital.

Diagnosis: While the etiological rôle of the sporothrix in this case cannot be considered to have been proven, still the weight of evidence seems to be in favor of a systemic infection by this organism. Against the diagnosis of a mycotic infection are the following points: (1) No apparent response to the potassium iodide treatment from the time of isolation of the organism. The lesion on the face became worse. In answer to this objection it is quite possible that the small doses of potassium iodide received during the year and a half of specific treatment previous to the isolation of the sporothrix, may have developed in the patient a tolerance to the drug. The organism was isolated while the patient was receiving 45 grains of potassium iodide a day. (2) Sporothrix infections very rarely produce cachexia and anæmia. That they do so in a small percentage of cases is, however, acknowledged by Beurmann. Tuberculosis and malignant disease were thought to have been excluded. The patient had received very thorough antisyphilitic treatment. Blood examinations excluded the graver anæmias. (3) Blood culture was negative. (4) The agglutination and complement fixation reactions were negative.

As regards the blood culture and these different reactions their presence in the severer sporothrix infections with regard to the different stages of the disease is not an established fact.

For help in the isolation and identification of the organism I am indebted to Dr. J. C. Torrey, and for the photographs and drawings to Mr. Bosse.

HISTOLOGICAL CHANGES IN THE ISLANDS OF LANGERHANS IN DIABETIC ANIMALS

F. M. ALLEN, M.D.

A short talk like this is no occasion for review of the literature or for theorizing. What I shall say will be meant simply as an explanation of a few slides showing the changes occurring in the islands of Langerhans of diabetic animals.

At a very early date clinical findings pointed to the pancreas in connection with diabetes. Later, the study of these gross alterations of the organ was overshadowed by the microscopic study, and there arose the so-called insular hypothesis of diabetes, with which the name of Opie is especially connected.

On the experimental side, as early as 1686 Brunner attempted the extirpation of the pancreas; but for over two hundred years attempts to produce experimental diabetes failed, supposedly because of incomplete pancreatectomy. Then v. Mering and Minkowski succeeded in the complete extirpation of the gland and demonstrated the severe diabetes which results. Shortly thereafter, Sandmeyer produced the form of diabetes known by his name, by removing most of the pancreas, leaving a small remnant isolated from the bowel, with part of its blood supply ligated. The atrophy of such a remnant gives rise, after a variable period of time, to a chronic, fatal diabetes. In both these forms of diabetes histological study is impossible. Various experiments have been performed with a view to studying the influence of the islands and the acini of the pancreas separately. The ducts have been ligated and the acinar tissue has been allowed to atrophy, while diabetes has apparently been prevented by persisting islet tissue. Various attempts have been made to produce specific injury of the islands, leaving the acini intact, but these have heretofore failed.

In this connection I wish to mention a third form of diabetes which results from partial removal of the pancreas without disturbance of the duct communications of the remnant. When conditions are thus kept simple, it is found that the removal of large portions of pancreatic tissue produces a lowering of the animal's sugar tolerance. When enough tissue is removed, diabetes results, which runs a chronic course extending over months and ends fatally, presenting, on the whole, a satisfactory imitation of human diabetes. In examining the organs and especially the glands of internal excretion of these animals after death, everything is found normal except the pancreas. Here the acinar tissue is normal, but the islands of Langerhans show specific changes. These changes may be summarized as vacuolization and loss of cytoplasm, pyknosis of nuclei and finally disappearance of cells and of islands. These changes ordinarily take place simultaneously throughout all the islands of the pancreatic remnant, although in some cases slight differences of degree may be observed between different islands or between different cells of the same island. Control experiments have been made to test the specificity of these changes in diabetes. By experiments with feeding and injection of various sugars, it is found that these changes are not the result of hyperglycemia. They also do not result from the Bernard puncture of the medulla, from injections of adrenalin, from phlorhizin poisoning, nor other conditions of non-diabetic glycosuria. Also, the operation of itself does not produce these changes. If the operation is performed leaving barely enough pancreas tissue to prevent diabetes, this exhaustion of the islands does not result. Likewise, the influence of diet can be demonstrated. If a certain fraction of tissue is removed, the presence or absence of diabetes may depend upon the diet. If such an animal is fed so as to produce diabetes, this diabetes then runs the usual course, and the typical changes in the islands occur. If the animal is fed so as to avoid diabetes, the changes in the islands are absent.

The relation of these findings to changes in the pancreas of human diabetics is preferably left to those pathologists who work with human material. It is possible that the "hydropic degeneration" described by Weichselbaum may be analogous. There is an interesting case reported by Ghon and Roman (*Prag. med. Wchnschr.*, xxxviii, No. 38, 1913) of an idiot boy of thirteen years who died of diabetes. At autopsy it was found that only the ventral "anlage" of the pancreas had developed. In the small mass of pancreatic tissue present, the islands were found to show exhaustion of the cytoplasm.

Discussion:

Dr. Levin: Dr. Allen states that when he removes three fourths of the pancreas the animals do not get diabetes and the islands remain normal. When four fifths of the pancreas is removed the animals become diabetic and the islands appear abnormal. I should like to know whether in the first class of cases all the islands remain normal and in the second class all become abnormal or do some of the islands of the first series become abnormal and those of the second series remain normal. I should also like to know whether the islands of the first series will become abnormal when the animals are treated with phloridzin after the operation.

Dr. MacCallum: I have been interested in this subject for a long time. I recall one case, at least, in which some of the changes described by Dr. Allen seemed to occur. It was a case of a child of fourteen years who died in diabetic coma. At autopsy the pancreas was nearly full size, but the islands of Langerhans presented certain changes, chiefly vacuolization and hyaline degeneration. In some cases of diabetes there seems to be a hypertrophy of some of the islands, indicating an effort of some cells to compensate for others. In one or two such cases I have observed that the cells of the islands were arranged in more or less transverse columns. I was not certain as to what the nature of this process was. In the effort to settle the question of the relation of the islands to glycosuria, we performed the following experiment: We ligated one lobe of the pancreas of a dog and removed the remainder of the pancreas. There was a slight glycosuria which disappeared. The dog was left for a year. The ligated remnant of the pancreas was removed. The dog died of glycosuria. The tissue removed was found to be a thin film of transparent tissue resembling in no way normal pancreas. Sections, however, showed that the islands of Langerhans were present. This experiment was repeated using a guinea-pig, and the tissue was stained with the Bensley stain, which showed that only the islands of Langerhans had survived. These two experiments seemed to me to offer very good evidence in favor of the island theory.

Dr. Allen: Dale's work was in connection with the idea of possible transformations occurring between insular and acinar tissue in the pancreas. A number of authors, especially Laguesse, have upheld the view that at any time fully developed acini may change into islands, or islands may change into acini, under the influence of various functional conditions. At present the best evidence is against this view; and especially the work of Bensley with differential staining methods seems to prove conclusively that such transformations do not occur. The Islands of Langerhans are not fixed and unchangeable structures like the glomeruli of the kidney, but regeneration of both acini and islands is by proliferation from the ducts, not by mutual transformations.

I have not seen this exhaustion of the islands in any non-diabetic animal. When diabetes is produced, the island changes seem to go parallel with the course of the diabetes, less marked in the earlier and milder cases, more extreme in the severe stages. The impression is given that a small number of islands try to do the work of the normal number, and that they are exhausted and break down in the process. Sometimes they do seem to show attempts at compensation, and apparently in some cases this compensation is successful, because in some cases it is possible to produce a diabetes which is temporary. The effect of phlorhizin upon the islands of normal animals is negative. Experiments with phlorhizin in partly depancreatized animals are now in progress, and the results cannot yet be stated; but the indications are that they will be negative.

Concerning the effects of duct ligation, the present evidence is that this procedure merely prevents diabetes by interfering with absorption of food and causing the animal to emaciate. In this, as in other cases, if diabetes is absent, the specific exhaustion of the islands does not appear.

PRACTICAL APPLICATIONS OBTAINED FROM THE SCHICK REACTION

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The Schick reaction (1, 2) is probably known to many here, but that all may understand it, the following brief review is given.

The reaction depends on the local irritant action of minute quantities of diphtheria toxin when injected intracutaneously. If antitoxin is absent, or present only in very small amounts, insufficient for protection, a positive reaction will appear in twenty-four to forty-eight hours. This is characterized by a circumscribed area of redness and slight infiltration which measures from 1–2 cm. in diameter. It persists for seven to ten days, and on fading shows superficial scaling and a persistent brownish pigmentation. The amount of toxin injected as advised by Schick is 1/50 MLD. for the guinea-pig in .1 c.c. of normal saline solution. We prefer 1/50 MLD. in .2 c.c.

It is important to distinguish the true reaction from a pseudo-reaction, which is only found in a small percentage of older children and adults, who may have a large amount of antitoxin. These reactions are probably local sensitization phenomena of a protein character, since similar reactions can usually be obtained with dilutions of an autolysate of the Klebs-Loeffler bacillus in which no toxin is present. The pseudo-reaction can be distinguished clinically by the trained observer in practically all cases from the true reaction. It appears earlier, is more infiltrated, less sharply circumscribed and usually disappears in 3–4 days. It leaves only a faintly pigmented area, which soon becomes invisible.

TECHNIQUE

For the carrying out of the test it is essential to have an accurate syringe with a sharp but short-pointed, fine needle. The usual 1 c.c. "Record" tuberculin syringe with a fine platinum iridium needle answers the purpose well. A standard diphtheria toxin is diluted at first 1 : 10 in 0.5 per cent. phenol; this dilution will keep in the ice-box with little deterioration for at least two weeks. For use further dilutions are made in normal saline, of such strength that .1 or .2 c.c. contain 1/50 MLD. for the guinea-pig. This amount is injected intracutaneously on the flexor surface of the arm or forearm. The persistent pigmentation may make the forearm objectionable; in such cases the surface of the arm may be chosen.

Though the intensity of the reaction varies in different individuals, a well-marked redness indicates an almost complete absence of antitoxin. Faint reactions point to the presence of very small amounts of antitoxin, which are not sufficient, however, to certainly protect the individual against diphtheria. The Schick test can be modified by injecting more toxic solutions. In this way some information can be obtained of the amount of antitoxin in the lymph and blood, but the results are less accurate than in the old, more time-consuming method. To prevent the appearance of the reaction, according to Schick, at least 1/30 unit of antitoxin per c.c. of blood is required. This amount he considers sufficient to protect against diphtheria. In a child three years of age, weighing 35 pounds, we found that 10 units of antitoxin were sufficient to prevent the appearance of the Schick test when made twenty-four hours after the subcutaneous injection of antitoxin.

RESULTS OF TESTS

The Schick reaction was carried out during six months on all patients entering the scarlet fever pavilion of the Willard Parker Hospital. In all, 700 patients were thus tested, and of these, 400, or 57 per cent., gave negative reactions. At first all cases were immunized but later only cases giving positive reactions; those giving a negative reaction while receiving no toxin-antitoxin mixture were carefully observed. Although more than a quarter of the negatively reacting patients became bacillus carriers during their stay in the wards, no cases of clinical diphtheria developed among them.

The remaining 300 patients who gave positive reactions received in practically all cases some form of toxin-antitoxin mixture. Among those who reacted insufficiently to the attempt at active immunization with toxin-antitoxin mixtures 42 developed clinical diptheria; of these, 20 were nasal, 20 tonsillar and 2 laryngeal diphtheria. The great majority of the cases were of a mild type.

According to ages, the number of reactions obtained were as follows:

Our Results (Drs. Zingher and Serota)

Age	Total	— Schick	+ Schick	Per Cent. + Schick
6 months to 1 year	5	3	2	40
1 to 2 years	40	14	26	65
2 to 4 years	145	48	97	66.8
4 to 6 years	142	71	71	50
6 to 8 years	94	61	33	35·1
8 to 15 years	150	111	39	26
15 years and over	124	93	31	25
	700	400	300	43

Schick's Results

Age	Total	— Schick	+ Schick	Per Cent. + Schick
Newborn	291	275	16	7
1st year	42	24	18	43
2 to 5 years	150	55	95	63
5 to 15 years	264	133	131	50
	747	487	140	34·9

The percentage of individuals susceptible to diphtheria is greatest, therefore, between the ages of one to four years. It is less during the second six months of life and less in older children and in adults. These results closely approach those obtained by Schick, whose table shows positive reactions in 7 per cent. of the newborn, in 43 per cent. during the first year of life, in 63 per cent. between two and five years and 50 per cent. between five and fifteen years. In adults the positive reactions were not more than 10 per cent.

During systematic testing by us at the Willard Parker Hospital and by Dr. Rosenberg elsewhere of groups of children according to families, we were impressed with the frequency with which all the children of the same family gave a similar reaction. If variations were found, the younger children always gave the positive reactions. If the youngest child had a negative reaction all the older children were also negative. On the other hand, if the oldest child in a family gave a positive reaction, the younger children with very few exceptions showed positive reactions. These striking facts lead us to believe that there are other factors in addition to infections with the Klebs-Loeffler bacillus (v. Behring, Kleinschmidt) which give rise to the presence of the so-called natural antitoxin. The large amount which is present in some of the cases is hard to explain; for example, two young children, six and one half and three years of age, who had no history of clinical diphtheria, showed respectively fifteen and nineteen units of natural antitoxin per c.c. of serum. The large proportion of adult persons having antitoxin adds to the difficulty of considering the natural antitoxin as due to a previous infection as a case or as a carrier. In a recent investigation by v. Groer the characteristics of the antitoxin present in persons who had never had diphtheria as found in the newborn appears to be identical with that due to immunization.

With this test Schick attempted to place on a more rational experimental basis some interesting clinical problems in connection with the prophylactic and therapeutic dosage and mode of administration of antitoxin. By making the test at regular intervals previous to the injection of antitoxin, he found that neutralization of toxin in contact with tissue cells was still possible up to a certain number of hours. This was demonstrated by the partial or complete suppression of the reaction. The efficiency of the injected antitoxin depended on the mode of administration and the size of the dose.

We carried out some parallel experiments attempting at the the same time to see approximately how much more effective the same dose of antitoxin was when given intravenously than when given subcutaneously or intramuscularly. The Schick test was made six hours, four hours and two hours before and at the time of the injection of the antitoxin. Comparing the results obtained from 100 units given intravenously with those obtained when the same amount was injected subcutaneously, we found that when injected intravenously the antitoxin weakened the reaction made two hours before and prevented the appearance of the simultaneous reaction; given subcutaneously, no effect was noted after 100 units on the two-hour reaction, and little, if any, on the simultaneous test. With 1,000 units given intravenously even the six-hour reaction was considerably weakened, though not entirely suppressed. The four-hour reaction appeared very faint, while the two-hour reaction was negative. 1,000 units given subcutaneously, weakened the reaction made two hours before, while the simultaneous reaction was just visible. The same amount (1,000 units) given intramuscularly had a somewhat stronger effect than the subcutaneous injection, and prevented the appearance of the simultaneous reaction.

To obtain the same effect on the Schick reaction with the subcutaneous as was obtained with the intravenous injection of antitoxin, from ten to twenty times the amount was needed; 20,000 units given subcutaneously were able to affect the six-hour reaction to the same extent as 1,000 units given intravenously. In very toxic forms of diphtheria, therefore, where a large amount of toxin has already come in contact with the cells of the body with a resulting greater or less amount of fixation, large doses of antitoxin (10,000 20,000 units) should be given intravenously to neutralize as much as possible the effect of such partially-fixed or contact toxin. No further injections are necessary.

The application of the Schick test to patients that have had diphtheria has revealed some interesting points in connection with the type of immunity which they develop after the disease.

We attempted to trace a number of the patients who had definite tonsillar exudates with positive cultures. 32 such patients who had been treated at the Willard Parker Hospital 3–4 months before, were tested by Dr. Rosenberg during an investigation of discharged contagious disease cases. 19 of the 32 gave positive Schick reactions while 13 were negative.

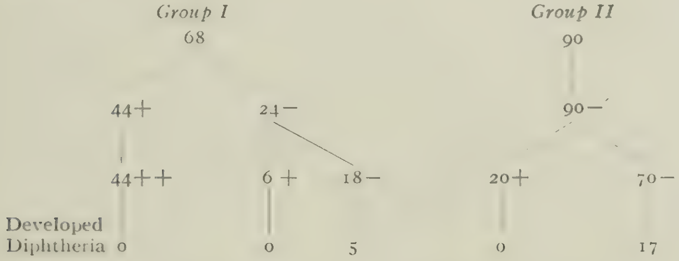
15 children were also tested at the Howard Colored Orphan Asylum, King's Park, L. I. 7 of the 15 had diphtheria about one year ago; of these 5 gave positive and 2 negative reactions; 8 cases had had the disease about 4 months ago, and of these 7 gave positive and one a negative Schick reaction.

Dr. Bennett, of Greenwich, Conn., tested 2 children, one 2 weeks and one 3 months after having clinical diphtheria. No antitoxin was given to the children and the disease ran a mild course. It was interesting to note that both children gave positive reactions. A nurse was also tested who had diphtheria about a year ago and she also showed a positive reaction.

The chronic tube cases who have been at the Willard Parker Hospital for more than a year, show, as a rule, a negative reaction. Patients who had diphtheria recently (2–3 months) frequently give a positive reaction which becomes negative if they remain for another 5–6 months. One child who had diphtheria in November, 1913, gave a positive reaction in January, 1914, and a negative reaction in August, 1914. Three other children who had diphtheria in March, 1914, were tested about the middle of August, 1914. One gave a negative, one a moderately positive and one a strongly positive reaction. They were retested 2½ months later, and the following results were noted. The negative case remained negative; the patient that had given a moderately positive reaction showed now a very faint reaction, while the strongly positive case gave a much weaker reaction at this time. When we tested again two months after that (eight months after illness) all these patients gave negative reactions and blood examinations showed presence of antitoxin.

The absence of antitoxic immunity in a large proportion of individuals who have had diphtheria as seen at all tests in the children who received no therapeutic antitoxin injections and in tests made after the disappearance of the horse antitoxin in those receiving injections leads us to assume that diphtheria patients develop as a rule an antibacterial immunity, which is associated in only about one third of the cases with an antitoxic immunity. Chronic reinfections with the Klebs-Loeffler bacillus, as seen in children who remain a long time in the diphtheria wards, finally lead in a majority of the cases to the production of an antitoxic immunity.

These results obtained in children after an attack of diphtheria are very significant in connection with the results which we obtained in the active immunization with mixtures of diphtheria toxin and antitoxin. The following tables summarize these results:



Presence of antitoxin, + ; absence of antitoxin, — .

In Group I, 68 patients were actively immunized. Of these, 44 had natural antitoxin and all gave a decided increase in antitoxin content after the injections; none developed diphtheria. Of the remaining 24 patients who had no perceptible natural antitoxin, 6 gave rise to antitoxin production, while 18 failed to respond. Of the 6 no one, of the 18, 5 developed clinical diphtheria.

In Group II, 90 patients were selected who had no natural antitoxin, as shown by blood examination and by a positive Schick reaction. Of the 90, 20 responded to the injection of toxin-antitoxin with the production of sufficient antitoxin to protect against diphtheria; 70 failed to respond, though some gave a fainter Schick reaction than they had shown before the immunization. Among the 20 no cases of diphtheria developed; of the 70 who produced no antitoxin 17 developed clinical diphtheria 12 days and more after the injection.

Active immunization, therefore, with mixtures of diphtheria toxin and antitoxin also fails to give rise in over 75 per cent. of non-immune patients to a sufficient antitoxin production to suppress the Schick reaction.

The absence of antitoxin in about two thirds of cases who had diphtheria and the inability of susceptible patients to respond to active immunization in an even larger proportion are indications which point to a possible value in the use of bacillary vaccines, either with or without antitoxin. Experimental work is now in progress at the Research Laboratory to test the value of such vaccines in animals and man.

The presence or absence of antitoxin in a case of suspected diphtheria was used by Schick as of value in diagnosis. We have also used it in the diagnosis of clinically doubtful nasal diphtheria. With a purulent or sanious nasal discharge showing the Klebs-Loeffler bacillus it is difficult to decide whether the case is a carrier or a beginning diphtheria. A negative reaction excludes diphtheria, while a positive Schick reaction leaves the diagnosis of diphtheria still a probability. A case of tonsillitis due to streptococci which was also a carrier of diphtheria bacilli, would by the use of the culture alone be thought to have diphtheria and in danger of extension of the disease. A negative Schick reaction would indicate the case to be simply a carrier and in no danger from the effects of the diphtheria poison.

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THE IMPORTANCE OF LIPOIDS FOR THE ADRENAL GLAND*

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Since the investigations of Adami and Aschoff, the significance of the cholesterin metabolism for physiology and its importance in pathological changes has played an important rôle in scientific investigations. The relationship of lipoids in general and of cholesterin in particular to the so-called glands of internal secretion, especially the cortex of the adrenal gland, has been much emphasized. Two diametrically opposed schools have developed in regard to this relationship, the first, the so-called French school composed of Chauffard, Grigaut and their pupils, supported by Albrecht and Weltmann in Germany, maintaining that the adrenal cortex manufactures cholesterin, while the other school, consisting of Landau, Wacker and Hueck, and Aschoff, regarding the cortex as merely a storehouse for lipoids.

Of the greatest importance are the investigations of Dorée, Ellis, Fraser and Gardner who demonstrated that the cholesterin equilibrium is maintained through the food intake, and that the fully developed chick contains only as much cholesterin as the egg. Pribram, Fraser, Gardner, and Wacker and Hueck showed that an increased cholesterin content of the diet manifests itself by an increase in the blood cholesterin and in the cholesterin of the adrenal cortex. Kusumato showed that 70 per cent. of the ingested cholesterin fails to appear in the feces. Ignatowsky, Stuckey, Wesselkin and Fahr demonstrated by feeding a cholesterin-rich food, that this substance is deposited in various tissues.

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* This work was carried on in the University of Freiburg, under the direction of Prof. L. Aschoff and his assistant, Dr. Landau, both of whom I desire to thank for their interest and assistance.

especially the aorta, adrenal cortex, liver, spleen, bone marrow and lymph nodes. Anitschkow, Chalotow, Wacker and Hueck, Weltmann and Biach, and Aschoff, by feeding pure cholesterin or cholesterin esters obtained similar results.

A critical review of these facts alone suggests the improbability of there being a real synthesis of cholesterin in the body. However, many authors, especially those constituting the school of Chauffard, have pointed out that in certain physiological and pathological conditions, independent of diet, there is a hypercholesterinemia with a hypercholesteratosis of the adrenal cortex; namely, atherosclerosis, chronic nephritis, diabetes, gout and jaundice and during pregnancy and the puerperium.

Grigaut divides all cholesterinemic states into two classes; First, those caused by retention, as in icterus; second, those caused by hypersecretion of the adrenal cortex and corpus luteum, as in atherosclerosis, etc. He supports this theory with the following facts: First, the cholesterin content of the blood and of the adrenal cortex run parallel; and secondly, by the results of adrenal extirpation experiments on dogs, which are cited below. The first of these arguments, namely the parallelism between the cholesterin content of the blood and the adrenal, may be used equally well by the second group of investigators who regards the cholesterin content of the adrenal as secondary to that of the blood. Also, as Landau pointed out, a hypercholesterinemia occurs in such a large group of pathological and physiological conditions that to regard a hypersecretion of the adrenal as the etiological factor in each would be improbable.

The experiments of Bacmeister and Havers have recently shown that the hypercholesterinemia in pregnancy is not due to a hypersecretion but to a retention. The pregnant dog with a biliary fistula during the first three weeks of pregnancy responds to any increase of cholesterin in its diet by an increased excretion of cholesterin in its bile. After the third week the cholesterin content of the bile falls and can not be influenced by diet, a marked hypercholesterinemia resulting. However, with the birth of the young there is a sudden and rapid excretion of the retained lipoids through the bile.

I shall now pass to the unilateral and bilateral adrenalectomy experiments of Grigaut. Dogs from whom one adrenal was removed showed at first no increase in the cholesterin content of the blood. After a latent period a hypercholesterinemia developed which reached its maximum on the twelfth day and returned to normal by the twenty-first day. After bilateral adrenalectomy he found no variations of the blood cholesterin. Grigaut explains these results by offering the theory that a latent period was required by the remaining adrenal to hypertrophy and hypersecrete.

From these experiments one can not say whether the hypertrophy of the adrenal precedes the hypersecretion as is assumed by the French school. Furthermore, no examination of the bile was made in these animals, and to control these experiments it was essential that both blood and bile should be examined.

The cholesterin metabolism of all carnivora and omnivora is so active that any increase in cholesterin content of their food manifests itself by increased excretion through the bile with little or no change in the blood cholesterin. No one has succeeded in producing artificial atherosclerosis in dogs by cholesterin feeding. Anitschkow has succeeded in producing it in rabbits. Herbivora in general have a very sluggish metabolism, little cholesterin being found in the bile. For the above reason rabbits were chosen for the following experiments. In order to avoid possible complications of pregnancy and to exclude any possible corpus luteum effects, male rabbits were selected. The rabbits were kept on a constant diet and blood taken at the same time each day in order to exclude dietetic increase. The methods used for chemical determination were the Autenrieth-Funk methods and the Windaus. The Autenrieth-Funk method requires only 2 c.c. of blood, and this method made the experiments possible as frequent examinations were essential.

The following necessary controls were made:

1. To determine the normal cholesterin content of rabbit's blood and its variability. With different animals variations from 40 to 80 mg. per 100 c.c. of blood were found. However,

with the same animal the variations are small, as for example, an animal observed over three months showed only a difference of 15 mg. between the lowest and the highest determinations.

2. The cholesterin content of the adrenal. Each adrenal was examined separately. They showed small individual variations, the normal quantity being about 11 to 13 per cent. of the dried gland.

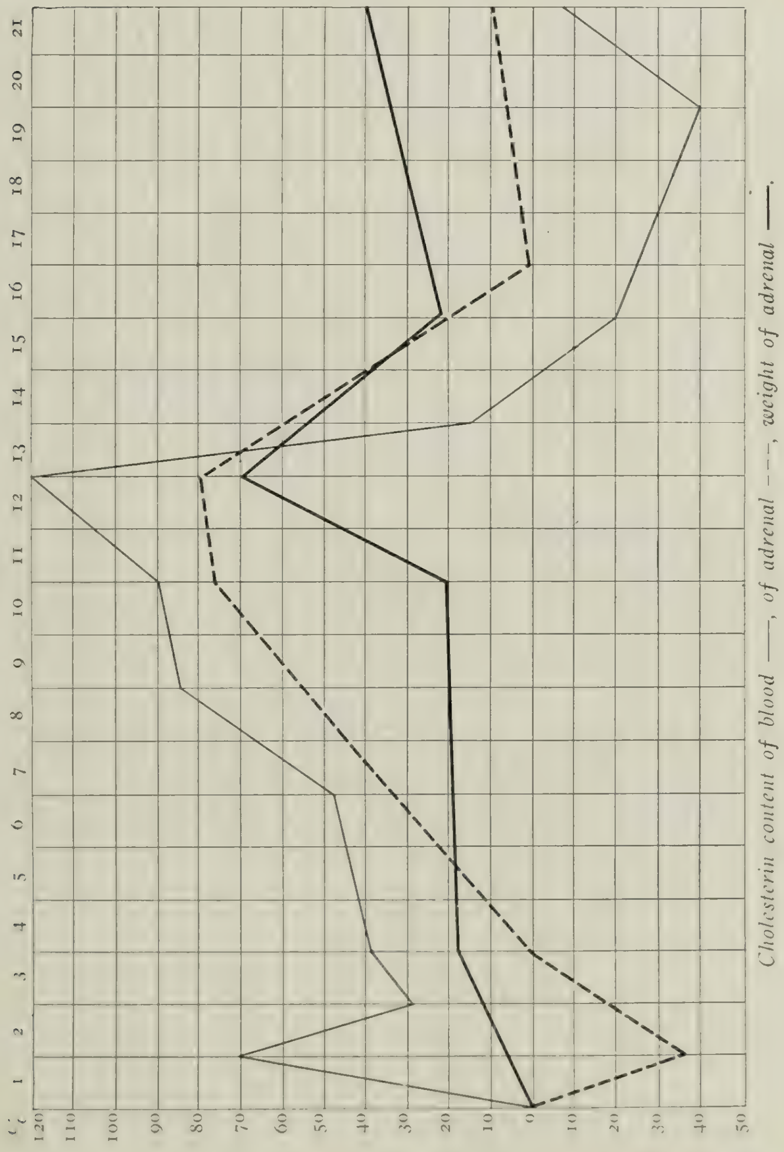
3. The cholesterin content of normal rabbit's bile. Rabbit's bile contained only traces of cholesterin varying between 5 and 15 mg. per 100 c.c.

4. Effect of feeding cholesterin upon the cholesterin content of rabbit's bile and blood. By feeding daily 0.4 gm. of cholesterin a hypercholesterinemia can be produced. The bile of these animals, however, contained only traces of cholesterin, until a certain grade of hypercholesterinemia is reached varying between 100 and 150 mg. per 100 c.c. As an example, a rabbit which had 48 mg. of cholesterin per 100 c.c. of blood received cholesterin for two weeks. Its blood cholesterin reached 110 mg. per 100 c.c., while its bile showed normal quantities, 15 mg. per 100 c.c. A rabbit fed fifty-nine days showed 364 mg. per 100 c.c. in its blood, while in its bile 160 mg. per 100 c.c. were found. Albrecht and Weltmann have claimed that there is a difference in principle between the cholesterin metabolism of herbivora and carnivora, claiming that herbivora excrete no cholesterin through the bile. The error lies in the methods used. There is no difference in principle, only a difference in degree in the activity of the liver as a filter. Because of this slow excretion of cholesterin through the liver, and because any increase of cholesterin in rabbits shows itself by a hypercholesterinemia before any increase shows itself in the bile, rabbits were the ideal animals for these experiments.

5. The influence of the operation and the narcosis on the cholesterin metabolism. A rabbit was given ether anesthesia for an hour and its blood cholesterin determined for ten days. A variation of 6 mg. per 100 c.c. was found during the entire period. Novocain-morphine anesthesia produced no effect on the choles-

TABLE I

Unilateral Adrenalectomised Rabbits



Cholesterin content of blood ——, *of adrenal* – –, *weight of adrenal* ——.

terin content of the blood. Decapsulation of the right adrenal under ether anesthesia and of the left under novocain-morphine anesthesia caused only a slight variation.

The experiments were performed as follows: Either under ether or novocain-morphine anesthesia the right or left adrenal was removed through a laparotomy incision. The blood was examined daily, and at different intervals varying from one day to twenty-one days, the animals were killed. The adrenals were examined chemically and morphologically; the remaining organs were also examined chemically and morphologically.

Table I represents a composite picture of the variations of the cholesterin content of the blood, the cholesterin content of the remaining adrenal in comparison with the removed gland and the weight of the remaining adrenal. From the table it will be seen that in the first twenty-four hours there is a sharp rise of the cholesterin content of the blood in contradistinction to the latent period found by Grigaut in dogs, followed in the next twenty-four hours by fall, and then a steady gradual rise to the twelfth day followed by a sudden fall to below normal on the fourteenth day, reaching its lowest point on the nineteenth, approaching normal again on the twenty-first day.

The examination of the bile for the first twenty-four hours shows a slight increase in cholesterin with a larger increase for the next twenty-four hours, corresponding to the fall in blood cholesterin, then a decrease until the twelfth day, followed by a large increase on the twelfth and thirteenth day, and finally a return to the normal amounts.

The weight of the adrenal increases for the first twenty-four hours, remaining fairly constant until the tenth day, and then increases to its maximum on the twelfth day followed by a gradual fall, but remaining above normal; then a secondary rise. It appears as if the hypercholesterinemia develops before the adrenal hypertrophies and the adrenal follows behind the hypercholesterinemia which in itself speaks against Grigaut's hypthesis. However, the cholesterin content of the remaining adrenal sinks below normal for the first twenty-four hours, rising to the normal on the third day, and then runs parallel to the content of cholesterin in the blood. Here also it appears that the increase of cholesterin in the adrenal follows the blood cholesterin and does not precede it.

The histological picture of the remaining adrenals shows a hypertrophy. The glomerulosa is hyperplastic, the cells of the fasciculata because of their increased lipoid content, larger and because of this their radial structure shows less distinctly. This picture is identical with that described by Sternberg in a study of the hypertrophy of the glands in pregnancy.

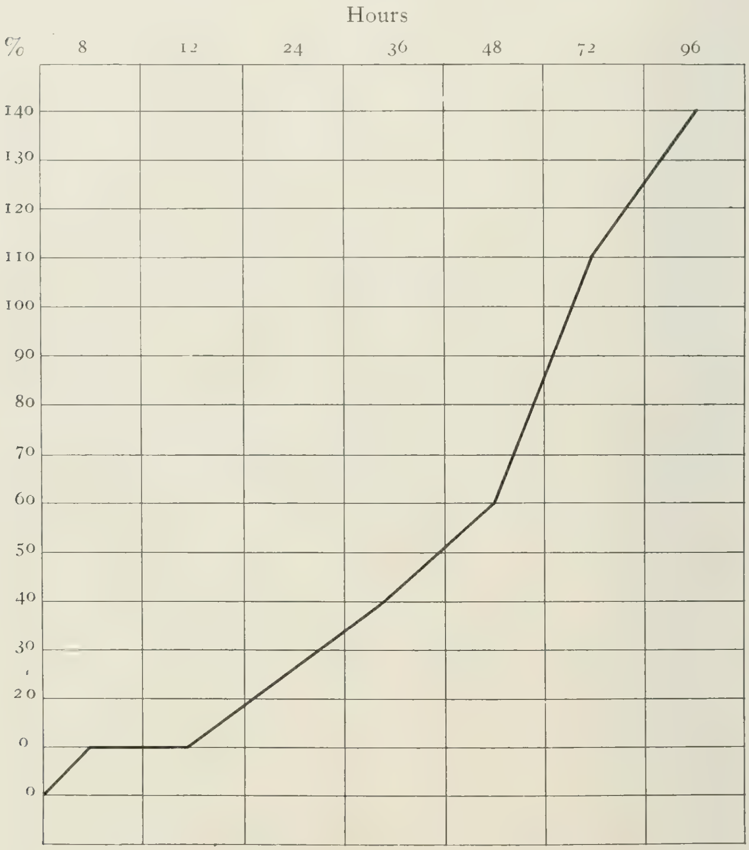
In general, after unilateral suprarenectomy there is the development of a hypercholesterinemia, a hypertrophy of the remaining adrenal, while the cholesterin content of the adrenal first falls and then runs parallel to the blood cholesterin. How can one explain the increase of the blood cholesterin and the fall of the content of the adrenal? Is the adrenal pouring its cholesterin into the blood stream or manufacturing cholesterin for the body? Anesthesia in general tends to exhaust the lipoid content of the adrenals. From these unilateral experiments alone one can not say which is the primary factor.

It was therefore necessary to perform the experiment of bilateral adrenalectomy and I shall discuss the results of unilateral and bilateral adrenalectomy together.

The removal of both adrenals was successfully accomplished in eight animals, the animals living from four hours to four days. Three of the animals showed small accessory adrenals. A blood curve of one of these animals is shown (Table II). The other animals showed, two hours after the operation, a marked increase of cholesterin in the blood, a rise which is sudden and increasing until the animal dies. The rise is greater in proportion and far more rapid than that following unilateral adrenalectomy. So one finds that in an animal without adrenals a more rapid and disdistinct hypercholesterinemia results than in an animal with one adrenal. The animals with accessory glands show a slight variation. The increase in blood cholesterin is not so rapid. In general, one may say, the more adrenal tissue remaining the less rapid is the development of the hypercholesterinemia.

TABLE III

Bilateral Adrenalectomized Rabbit. Accessory Adrenal. Weight = 38 mg. Bile = 76.8 mg., cholestetrin per 100 c.c.



Blood cholesterin, ——.

One naturally asks where the large increase of cholesterin comes from. Is this cholesterin being poured out from the adrenals at the time of operation? This is not possible as both adrenals contained about the normal quantities of cholesterin after removal, and secondly, the increase in the blood is at least 150 mg. of cholesterin, while normally the adrenals themselves only contain 10 to 20 mg. The increase can not be explained on the food intake as the animals are too ill to eat and live too short a time. However, these animals lose a large amount of body weight, at least one quarter to one third. The general catabo processes and the breaking down of fatty tissues in particular must explain this phenomenon. Furthermore, the bile contains large quantities of cholesterin in contradistinction to the normal rabbit's bile as is seen in starvation experiments where a similar catabolic process is taking place. The organs rich in cholesterin show no decrease in their cholesterin content. The brains of three of these animals were examined and gave normal figures. The liver shows an increase in its cholesterin content corresponding to the increased excretion in the bile.

The next question which develops is how a cholesterin equilibrium is reestablished. Here the regulating power of the liver comes into consideration. The sudden excretion of cholesterin in the bile corresponding to the decline in the blood shows how an equilibrium is reestablished. The finding of large quantities of cholesterin in the bile speaks for a coincident or previously existing high grade of cholesterinemia. In other words the increase of blood cholesterin is not the only criterion of a hypercholesterinemia. This fact is even more important in carnivora which normally excrete large quantities of cholesterin in the bile and explains the seemingly divergent results obtained by Grigaut in dogs after unilateral and bilateral adrenalectomy.

Especially interesting is the clinical behavior of these animals after adrenalectomy and above all the cause of death. A small percentage of unilateral adrenalectomized animals die from four to nine days after the operation and show the characteristic type of death of the bilateral adrenalectomized animals. This phenomenon is well known and has been described by Tizzoni, Hultgrin and Anderson, etc. The suddenness of death is especially interesting. They lose from 300 to 600 grams of body weight. They appear less lively than animals who survived the operation. The weakness increases so that finally the animals can not support themselves and appear as if paralyzed. The breathing is rapid, the heart's action increased, the pupils are normal (not dilated), the blood vessels are empty and it is difficult to obtain blood. In this condition they die, or shortly before death convulsions occur. No pigmentation was noted in any of these animals, but Tizzoni, who allowed his animals to live 200 to 300 days, noted the development of marked pigmentation.

Autopsy findings on these unilateral and bilateral adrenalectomized animals show no cause for death. However, if one examines the remaining adrenal one finds a characteristic hypertrophy microscopically and macroscopically, and the lipoid content of this organ is decreased even to practical exhaustion. These animals develop a lower grade of hypercholesterinemia than the unilateral adrenalectomized animals who do not die immediately and before death a decrease in the cholesterin content of the blood. The bile at the time of death shows only traces of cholesterin. We can thus say that these animals in contradistinction to the other unilateral adrenalectomized animals show before death only a moderate hypercholesterinemia and present a hypertrophy of the remaining gland with a cortex poor or exhausted in lipoids.

From these findings one can not maintain that the adrenal is producing the cholesterin, as is held by the Chauffard school. These experiments speak for the fact that the cholesterin is stored in the adrenal. Just as one can not speak of a production of cholesterin in the glands one can not speak of an esterization or de-esterization function of these glands, as in animals without adrenals the blood may show an increase in free cholesterin or an increase in cholesterin esters or show normal relationships between the two.

The above described experiments prove that the removal of the adrenals causes a disturbance of the cholesterin metabolism, and it follows that the adrenals not only store the cholesterin but use it for some purpose. If the cholesterin content of the remaining adrenal of the unilateral adrenalectomized animals be too small, despite the normal amount in the blood these animals die similarly to completely adrenalectomized animals. The important fact develops that the cholesterin in the adrenal cortex is of vital significance.

In human pathology, especially in Addison's disease, these experiments may have some bearing. According to Wiesel and others, Addison's disease is one of the chromophine system. The pathological changes in the adrenals have long been known. Investigations of Stewart and MacCallum have shown that pregnant animals survive bilateral adrenalectomy longer than non-pregnant animals. Hultgrin and Anderson demonstrated that castrated animals live longer than non-castrated ones. Stewart, Wacker and Hueck showed that before or after the operation of bilateral adrenalectomy, if cholesterin was fed, the animals live longer. Animals with accessory adrenals also survive the operation longer. The one point in common in all of these experiments is the hypercholesterinemia. In the animals with no adrenals, the organism attempts to compensate by a massive increase in the cholesterin content of the blood. On the other hand, animals not showing this hypercholesterinemia die as do animals with no adrenals.

Extirpation of the semilunar or solar plexus has proven ineffective in producing this picture or the death of the animals. Feeding or injection of adrenals has also proven of no service. Also all chromophine tissue has an adrenalin producing function. Therefore the extirpation of the adrenal causes above all a disturbance in the functions of the cortex, namely of the lipoid metabolism. The hypercholesterinemic animals die with exactly the same phenomena as those showing a normal cholesterin content of the blood. The main factor is apparently the absence of the cholesterin rich tissue of the adrenal cortex. The important point in Addison's disease would therefore appear to be not how much cholesterin is present in the blood but the absence of lipoid and especially cholesterin from the adrenals.

CONCLUSIONS

1. After unilateral adrenal extirpation in rabbits a rapidly developing hypercholesterinemia results, the other adrenal hypertrophies its cholesterin content first sinks and then runs parallel to that of the blood.

2. After bilateral adrenalectomy a more marked hypercholesterinemia develops. In suprarenectomized rabbits, as in other hypercholesterinemic states, in contradistinction to the normal the bile contains large quantities of cholesterin.

3. Many unilateral adrenalectomized animals without any recognizable causes for death show no or only a transient hypercholesterinemia and an exhaustion of the cortex in lipoids. These die with the same phenomena as bilateral adrenalectomized rabbits.

4. The adrenals can not therefore be regarded as producers of cholesterin in the sense of the French school.

5. In the animals without adrenals there is no disturbance of the esterization or de-esterization function; the theory of esterization or de-esterization function of the adrenals is unnecessary.

6. The adrenal cortex can not be considered as a source of the blood cholesterin. Its vital significance lies in its content of cholesterin and lipoids.

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Dr. A. M. Pappenheimer, *President*

MALIGNANT MELANOTIC TUMOR, PRIMARY IN THE ADRENALS

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The origin of melanotic tumors from tissues other than the skin and choroid has been considered highly questionable by so good an authority as Borst. Nevertheless, there have been reported from time to time, fairly well substantiated cases of melanotic growths arising from the adrenals. The number of cases in which the adrenal origin of the tumor is reasonably certain, is very small, and it seems desirable to place on record an additional example.

The patient, a man 50 years of age, entered the Presbyterian Hospital April 30, 1914.

CLINICAL HISTORY

Family History.—Negative.

Personal History.—Painter for 25 years. Gonorrhea and syphilis denied.

Past History.—Typhoid at 15 years. Painter's colic and blue line 12 years ago. Stopped painting 18 months and recovered. Otherwise well.

Present History.—Gradual onset four months ago with dull headache. For past year has had dull, cramp-like pains in stomach once or twice a day, not related to eating. No vomiting. A few days after headache began, had atack of fainting lasting 15 minutes. Then remained in bed because of weakness. For past three weeks legs have been weak. No wrist or foot drop. Headaches and stomach pains have continued irregularly. Bowels constipated. No disturbance of vision. Has lost some weight.

Physical Examination.—Well nourished. No dyspnea or cyanosis. Mentally clear. Head normal. Pupils small and equal, react normally. Arcus senilis. Upper teeth false, only stumps in lower jaw. Gums have receded and are soft. Marked bluish deposit in the gums at bases of teeth. Breath foul and tongue coated. Few large glands in submaxillary region. *Lungs* normal. *Heart:* Apex 4th space, 9 cm. to the left. No murmurs. *Blood Pressure* 142. *Abdomen:* Normal. Edge of liver indistinctly felt 3 cm. below costal margin. Spleen and kidneys not felt. All reflexes exaggerated. No œdema. No wrist- or foot-drop. *Superficial glands:* Mass in right axilla about 3.5 cm. × 2 cm. which feels like a mass of glands, is not tender, and freely movable. No other glands enlarged. There is peculiar blackish deposit in the skin below angle of right scapula. Lentigo over posterior chest.

May 1: Hgb. 75 per cent., W.B.C 12,800, Polys. 72 per cent.

May 2: Ophthalmoscopic examination negative. At 6:45 A.M. had many epileptic convulsions. Started with twitching in left arm and left side of face, then became unconscious and had clonic convulsive movements lasting 2–3 minutes. Very cyanotic. After convulsion perspired freely and was stuporous for about 2 hours, then irrational, complaining of something on back of his neck which should be turned off.

May 3: Phthalein test: Color appeared in 10 minutes. Total excretion 2 hrs. 21.7 per cent. Headache all day. Drowsy.

May 4: Lumbar puncture, pressure not increased. Leukocytes 2. Wassermann negative.

May 5: Blood urea 0.04 per cent. Lead present in urine. R.B.C. 4,438,000. No stippling. Hgb. 78 per cent.

May 6: Increasing stupor for 24 hours. Yesterday restless and severe headache. Involuntary discharge urine and feces.

May 7: Blood Wassermann negative. X-Ray of skull negative.

May 8: Phthalein test: Total 46.7 per cent. W.B.C. 20,500, Polys. 80 per cent., Lymphos. 19 per cent., Eosinophiles 3 per cent. No stippling of red cells. Lumbar puncture. Pressure not increased. Leukocytes 22, many r.b.c. Butyric acid test positive. Wassermann—0.1 c.c. Negative, 0.3 c.c. +, 0.5 c.c. ++. Lead present in urine. On May 7 temperature 102°, very stupid. Involuntary dejections. Muscles spastic.

May 11: May 12 very stupid with Cheyne-Stokes respirations. Brighter to-day, but irrational. No Cheyne-Stokes respirations. Muscles very spastic and reflexes exaggerated. Lumbar puncture: dry tap.

May 15: Lumbar puncture. Slightly turbid fluid under some increase of pressure.

Urine: Sp. Gr. 1013–1032, albumen absent three times, once a very faint trace. No casts.

Temperature: Between normal and 100°. Occasionally as high as 102°.

Blood Pressure: 94–166.

Autopsy May 16, 1914.

Body is that of an adult male measuring 166 cm. in length. The body is spare and poorly nourished. The visible mucous membranes are pale. Pupils equal. There is slight arcus senilis. There are no teeth in the upper gums. In the lower jaw, there are three incisors and one canine. The right middle incisor is missing. The gums are retracted about the teeth and spongy. They show a dirty bluish discoloration. Liver mortis is present over the back and dependent portions. There are numerous light brown spots scattered over the shoulders and back. Beneath the angle of the right scapula there is a pigmented nævus. Rigor mortis is present in all extremities. The muscles of the limbs are not noticeably atrophied; those of the trunk are dry and a trifle pale.

The peritoneal cavity is free from fluid, the serosa glistening and smooth. The omentum contains a moderate amount of fat and is not adherent. The appendix is directed upward behind the cecum. There are fibrous adhesions between hepatic flexure and fundus of gall-bladder. Diaphragm reaches the 3d interspace on the right side, 4th interspace on the left side.

The pleural cavities are free from fluid and adhesions. The pericardium contains a few c.c. of clear yellow fluid. Adherent to the entire mediastinal surface of the pericardium in its upper portion, there is a rounded mass about the size of a walnut, which on section is found to consist of soft grayish-red tumor tissue.

Left Lung: The left lung weighs 520 grams, and is very voluminous. The pleura is shiny and smooth. The fissure between the upper and lower lobes extends only half way to the anterior border. On section the lung is everywhere well aerated, though very œdematous and mottled here and there with a few darker reddish areas. The bronchi are somewhat congested and contain frothy fluid. The bronchial lymph nodes are small and pigmented. The pulmonary artery shows a few yellowish patches. There are a few small, circumscribed, homogeneous white tumor nodules scattered throughout the substance of the lung.

Right Lung: The right lung weighs 600 grams. The pleura is normal. There is on this side also an anomalous lobulation, the fissure between the upper and middle lobes being represented merely by a shallow notch at the posterior border. On section, the appearance is the same as that of the left lung; the œdema perhaps is slightly more marked. Small tumor nodules are found in both upper and lower lobes. None of these exceed a few mm.

in size. The bronchial lymph nodes are enlarged and deeply pigmented. Cross section shows them to contain definite nodules of tumor tissue.

Heart: The heart weighs 310 grams. The epicardium is smooth. There is a moderate amount of epicardial fat. Along some of the smaller branches of the coronary arteries, there are seen a few minute flat whitish nodules. The right auricle is normal. The foramen ovale is closed. The tricuspid ring measures 10.5 cm. in circumference. The valves are normal. The pulmonary orifice measures 72 mm. in circumference. Wall of right ventricle measures 4–5 mm. in thickness. Left auricle is normal. The mitral ring measures 11 cm. in circumference. The valves are delicate. The aortic orifice measures 76 mm. in circumference. There are atheromatous patches on the aortic surface of the mitral valve. The aortic cusps are a little thickened at their bases. The edges are fenestrated, but delicate and freely movable. The left ventricle measures 12–14 mm. in thickness. The heart muscle is soft, a little brownish, and on section shows a few darker red areas, but no scarring. The coronary arteries are delicate and free from atheroma.

Liver: The liver weighs 2,150 grams and measures 28 × 21 × 9 cm. The surface is smooth, mottled yellowish red with scattered single lobules of bright yellow color. On section, the organ is of normal texture, with distinct lobules having a reddish center and paler grayish periphery. Near the falciform ligament, in the left lobe, there is a well-circumscribed nodule about 1.5 cm. in diameter, not much raised above the surface, composed of soft grayish yellow tissue with areas of opacity and hemorrhages. There is also a larger group of nodules situated in the substance of the right lobe near the hilus.

Gall Bladder: The gall bladder is distended with dark thin bile. The mucosa is smooth.

Spleen: The spleen weighs 275 grams and measures 14.5 × 9 × 4.5 cm. The capsule is roughened in places. On section, soft, with very large irregular gray follicles. The pulp is mottled with small hemorrhages.

Pancreas: The pancreas is of normal color and texture, and free throughout from tumor tissue. The splenic artery is wide and tortuous. The splenic vein is empty.

After removing the intestines and stomach, the adrenals are seen to be replaced by large rounded masses of tissue, conforming in a general way to the normal shape of the adrenals, and everywhere enclosed by a thin capsule, so that neither the kidneys nor the adjacent cellular tissue are invaded by the growth. The two large adrenal masses together with the kidneys, ureters, pelvic organs and great vessels of the thorax and abdomen were removed en masse.

The renal veins and vena cava are found to be free from thrombi or tumor masses. Several veins leading directly into the tumor masses were also opened and found to be empty.

Left Adrenal: The left adrenal forms a more or less globular mass which measures 7.5 × 6 × 3.5 cm. It is very soft, almost fluctuating and on incising the mass, the tumor tissue bulges out of the tense capsule. It is extremely friable and soft, shows no tinct lobular structure. The surface of the cut section is in general grayish red, but variegated in as much as one can distinguish white areas having the consistence and color of brain tissue; with areas of cystic softening filled with fluid; looser grayish and grayish red portions and finally areas of hemorrhage. Just beneath the capsule there is a narrow strip of ochre-colored adrenal cortical tissue still recognizable, which can be traced over the anterior surface for a considerable distance. So far as can be determined, the tumor occupies therefore the medullary portion of the gland.

Right Adrenal: The right adrenal also is the seat of a tumor mass having the same general conformation and appearance as that described in the left. The mass on the right side measures 10 × 6 × 3 cm. It is composed of the same sort of friable tumor tissue as the left adrenal, but is slightly more hemorrhagic.

Kidneys: The right kidney measures 10 × 6 × 3 cm. The capsule is readily stripped. The surface is smooth and the cortex measures 5 mm. in thickness. Striations are distinct and even. The color is a uniform reddish brown, the consistence firm but not hard. The vessels are not prominent.

Left Kidney: The left kidney measures 12 × 5.5 × 4 cm. It resembles its mate in all respects.

Urinary Bladder: The mucous membrane is normal.

Prostate: The prostate is not enlarged, is symmetrical, of normal structure on section.

Testes: Slightly brownish, the tubules pull out with slight difficulty.

Œsophagus: Is normal in its lower portion, the upper part not examined.

Stomach: There is a circular constriction in the middle portion, due to muscular contraction. The mucosa is normal throughout, the folds marked only in the pyloric portion. There is a finger shaped diverticulum about 5 cm. in length and about the thickness of one's thumb, given off from the fundus, and directed upward and to the left. All three coats are apparently represented in the wall of the sac, but they are thinned out. The lining mucosa is not altered.

Intestines: The duodenum is normal. Small intestine: from the serous surface one can see and feel a number of irregular nodules scattered throughout the jejunum and the upper part of the ileum. These appear from without as irregular white areas, shining through the serosa and surrounded by injected venules. After opening the gut, the uppermost nodule is found about 5 cm. from the beginning of the jejunum. Here there is a submucous nodule about 2 cm. in diameter which projects in polypoid form into the lumen. It is entirely covered by intact mucosa, and on section appears as a circumscribed homogeneous soft white mass lying in the submucosa. From this point on, numerous similar metastatic nodules are found. The smallest ones appear merely as oval thickenings of the valvulæ. Large ones tend to become polypoid and one is distinctly pedunculated. The overlying mucous membrane is for the most part unaffected, but over some of the large tumors, there is distinct ulceration. The largest mass is found in the upper part of the ileum. It is oval in shape, and extends about two thirds around the gut. It projects like a large button or raised disc into the intestine. The center shows a ragged crater-like excavation, and the surface is strikingly pigmented yellow and black.[1]

The appendix is 12 cm. in length. The lumen is patent and the mucosa normal up to within 2 cm. of the tip, where it becomes suddenly obliterated. The large intestine is normal throughout and is free from tumor nodules. The rectum also contains no new growth.

Lymph Nodes: The mesenteric lymph nodes are not much enlarged, but some of them on section contain circumscribed tumor nodules. The retroperitoneal and hypogastric lymph nodes are also the seat of metastatic deposits, but are not much enlarged. The lymph glands at the bifurcation of the trachea form a packet of considerable size. On section they also contain circumscribed tumor nodules, standing out in sharp contrast to the anthracotic lymphoid tissue.

The organs of the neck, head and spinal cord could not be examined, permission being withheld.

Right Adrenal: Transection after hardening shows a continuous shell of cortex surrounding the entire tumor mass. This is thinned out to 1 mm. or less on the anterior surface, and is thickest at the upper and lower poles, where one may distinguish portions of the pigmented zona reticularis.

Left Adrenal: The left adrenal is also enclosed in a very thin shell of cortical tissue.

MICROSCOPICAL EXAMINATION

Tumor Tissue: With variations to be noted, the tumor tissue shows the same structure in the original growth and in the metastases. It is composed for the most part of round or polyhedral cells of rather large size having a round, occasionally slightly oval or indented nucleus which stains well and shows a finely distributed delicate chromatin network without a large or distinct nucleolus. In many places are found cells containing two or three nuclei and in a few places larger conglomerations of nuclei with a common protoplasmic syncytium. These giant cells may contain ten or twelve closely clustered nuclei. The cytoplasm in some places is very indefinite, pale, and finely granular. In other places the cell outline is more sharply defined. It is usually spheroidal, not polygonal, but not infrequently is drawn out into a short spindle.

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[1] A close examination of the intestinal nodules in the hardened museum specimen shows distinct patches of a grayish color scattered through the cream colored tumor tissue.

In the intestinal nodules the cells are somewhat larger and disposed in a sort of alveolar arrangement. Many of them contain a fine brownish granular pigment, the amount of which and the color vary in different cells. This pigment gives no iron reaction, is not removed by lipoid solvents, and resists decolorization by 5 per cent sulphuric acid and by strong alkalies. With polychrome methylene blue, it takes a deep greenish-blue color. The nuclei of these pigmented cells show curious degenerative changes. Many of them are shrunken and pyknotic. In others the central portion of the nucleus is clear and the chromatin lies against the nuclear membrane giving a ring or signet appearance. In some cells the degeneration is more extreme and the chromatin fragmented into deeply staining particles which are found also free between the intact cells or as inclusions within other cells. In the intestinal nodules there are scattered eosinophilic leukocytes between the tumor cells. These also show the nuclear pyknosis and fragmentation. The arrangement of the tumor cells is highly irregular. In many places they are loosely aggregated and not separated by a definite stroma, so far as can be judged in hematoxylin eosin stains. They may form nests of solid alveoli separated by loose strands of connective tissue. In still other places the individual cells are separated by an ill-defined finely fibrillar staining substance. In still other places, and this is especially conspicuous in the original tumor, the cells are grouped in a more or less radial fashion about thin-walled blood vessels. Between these mantles of intact tumor cells there are often extensive fresh hemorrhages. Large areas of necrosis, involving not only the tumor cells but the blood vessels and stroma as well, are present in many of the nodules, especially in the large metastatic growths in the bronchial lymph nodes. In other places the tumor tissue is rarefied by collections of fluid coagulum. Mitoses occur with moderate frequency.

Lungs: The capillaries are very congested and somewhat tortuous. The alveoli are for the most part empty but some of them do contain a loose exudate composed principally of polynuclear cells and of a little granular coagulum. The tumor nodules are well circumscribed, show no definite relation to blood vessels and are free from necrosis. With the immersion, isolated cells show diffuse brownish pigmentation.

Liver: Section passes through a large tumor nodule in which the tumor cells show a distinct perithelial arrangement with intervening areas of necrosis. It is surrounded by a dense fibrous capsule which sends septa between different portions of the tumor tissue. About the tumor nodule there is a tangential compression of the liver cells and a very striking dilatation of the capillaries with formation of large sinuses. There is also a definite œdema of the liver tissue and the accumulation of many wandering cells principally mononuclear but occasionally eosinophiles both in the distended capillaries and outside of the capillary wall between it and the compressed liver cells. Some of the liver columns show quite distinct accumulation of brownish granular pigment.

Spleen: The sinuses are very distinct from the rather fibrous pulp and are well filled with red cells. There are a few irregular hemorrhages. The Malpighian bodies are moderate sized, composed throughout of cells of the small lymphoid type. The trabeculæ and the blood vessels show nothing of note.

Kidney: There is a well-marked congestion of all the capillaries including those of the tufts. The glomeruli are normal. The epithelium of the convoluted tubules is somewhat swollen, with a distinct granulation of the protoplasm. The blood vessels show a slight increase in the adventitious coat, but are not otherwise altered.

Intestinal Nodules: Sections through various intestinal nodules show essentially the same picture. The tumor growths are found in the submucosa or in the loose tissue of the mucosa itself. The muscular coat in the larger growth is compressed but not infiltrated. One of the nodules has distinctly a polypoid form, and the mucous membrane continues for a considerable distance on the inferior surface of the polyp, but is necrotic over its convex portion.

Bronchial Lymph Glands: The bronchial lymph nodes are extensively replaced by tumor tissue. They show in addition a few old tuberculous foci. In the capsule of the gland are found irregular brownish concretions in some places surrounded by foreign body giant cells.

Prostate: The ducts are dilated and filled in many places with desquamating epithelium which is heaped up. In some of the tubules the heaping up of the epithelium is so marked that the lumen is practically obliterated. There is also a good deal of papillary infolding.

Myocardium: Aside from slight œdema and fragmentation of the fibers, is normal.

Stomach: Is normal.

Anatomical Diagnosis: Primary malignant tumor of adrenals, with metastases in liver, lungs, bronchial, peritracheal, retroperitoneal and mesenteric lymph-nodes, and in small intestine. Œdema of lungs. Brown atrophy of heart. Diverticulum of stomach. Localized chronic appendicitis with obliteration. Pigmented nævus.

DISCUSSION

The primary origin of the tumor from the adrenals in this case seems beyond question. In view of the ophthalmoscopic examination, it is unlikely that there existed a choroidal tumor, and there were no cutaneous growths aside from the small pigmented nævus, which showed no histological signs of malignancy.

In spite of the absence of pigment in the adrenal tumors themselves, the melanotic character of the growth is perfectly evident from a study of the intestinal metastases, in some of which the pigment is very abundant. One finds also occasional pigmented cells in the pulmonary and lymphatic nodules, but only after careful search.

The chemical reactions of the pigment, so far as they were studied, agree with those given by the normal pigment of the reticular zone. Lucksch[1] has studied the pigment in benign pigmented adenomata of the adrenal cortex, and obtained similar reactions. The crude tests which we have applied are at least sufficient to exclude an accidental pigmentation from previous hemorrhages, and to suggest that the pigment is elaborated by cells derived from the adrenal cortex.

The previously reported cases of melanotic tumors of adrenal origin are too few to justify an extended analysis. In four of the nine reported cases which I have been able to find, the data are too incomplete to make the adrenal origin of the tumors certain. Thus in a case mentioned by Orth,[2] and described by Döderlein,[3] there is no record of the eyes or skin, and no microscopical examination. Another case, reported by Davidsohn,[4] was later found by Marchand to have had a tumor of the choroid. The data in two other cases—one of Orth's and one published by Reimann[5]—are also too meager to justify conclusions.

The cases of Schweikert,[6] Davidsohn[7] (Case 2), Schmidt,[8] Schum,[9] and Molnar[10] are well-reported, and include references to the condition of the eyes. The tumors have all occurred in later life (except the case recorded by Molnar in a man of twenty-nine), and four of the five have been in males. In all the cases, there were secondary growths in the gastro-intestinal tract, particularly, as in our case, in the jejunum. The brain, lungs, lymph-glands seem also to be especially disposed, the liver metastases, in contrast with the general rule in melanotic tumors, few or entirely wanting. Cutaneous or subcutaneous nodules were usually present, and two of the cases showed growths in the bones.

Schum's case is interesting in that the original adrenal tumor was pigment-free, and only the metastases pigmented.

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ATYPICAL HEMOPHILIA

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The case which I wish to report this evening is that of a child five and a half years old, which suffers from hemophilia of a somewhat unusual type. In the first place this child, as far as we can judge, is the only one of the family who is a bleeder. In other words, it is a sporadic case and not one of the "family" variety.

Just a word about the history. The child is well nourished and the only other abnormal symptom or sign which he shows is a mild degree of ichthyosis. He has been an inmate of the Hebrew Infant Asylum for the past three years and has been under particular surveillance by me since last winter. During this period he has had many hemorrhages from minor cuts which he has had from time to time. He has frequent bleedings from the nose and occasionally hemorrhages beneath the skin, especially on the frontal bones following slight trauma.

As you all know, the pathogenesis of hemophilia is still by no means clear; it is thought by some to be due to a lack of prothrombin, by others to lack of thrombokinase. Whatever the assigned cause, there has been, I think, a general agreement that a lack of calcium does not play a part in the etiology. The case which I shall briefly report is interesting in that calcium does seem to play a rôle. The blood of this boy has been tested many times since last April, five months ago. In some instances the oxalated plasma was used and the clotting was found to be delayed. It varied between 58 minutes when it was first taken and 12 minutes in the later tests when the child was greatly improved in its general condition. The blood itself was likewise tested. For this purpose we have followed in a general way the simple technic recommended by Howell, which consists in dropping 10 drops of blood into a small vial and watching the process of coagulation. By both these methods the blood was found at all times to show a delay in coagulation. It is important to emphasize this point as it serves to differentiate hemophilia from purpura, which it might be thought this case represented. Another differentiation between these two conditions depends upon the fact that in purpura there is an essential weakness of the vessel walls. In purpura if you subject the vessels to an increased pressure, that is apply the *capillary resistance test,* which I have elsewhere described,[1] the vessels yield, whereas in hemophilia they do not show any weakness. In the case under discussion no petechial spots followed the application of increased pressure, so that we must regard this case definitely as a case of hemophilia.

The disturbance in calcium was demonstrated in several ways. Of course, the most direct and conclusive method of showing a lack of calcium in the blood is by quantitative analysis. Since the introduction of McCrudden's method, which enables one to determine the amount of calcium in as little as 5 c.c. of blood, it is possible to use direct methods for this purpose. Five tests of the blood were made. The accompanying table (Table I) shows the amount of calcium as calculated per 1,000 gm. of blood. This table shows five tests of normal blood made by means of the same method and one test of a case of hemophilia of the hereditary type.

TABLE I

Calcium Content of Blood (CaO)

Case of Sporadic Hemophilia	Normal	Typical Hemophilia
0.047	0.082	0.068
0.056	0.075	
0.058	0.079	
0.058	0.074	
0.062[1]	0.084	

[1] After giving calcium lactate.

A glance at this table shows that there was a lack of calcium in our case. This lack was particularly marked in the first test and became less in the later tests after the boy became better nourished and had gained considerably in weight. After the fourth test, calcium lactate was given in 30 gm. doses per day in order to discover whether by this means, the calcium in the blood would be increased. As will be noticed this resulted in a slight increase in the quantity of calcium. Previous to this test, the blood had increased to such an extent in its content of calcium it was hardly to be expected that the giving of this additional calcium would accomplish more than a slight increase. It may, therefore, be said from the result of this part of the investigation that the calcium was definitely found to be diminished. There seemed to be waves during which the calcium was higher and lower.

In addition to this direct test of the blood, experiments were carried out by means of adding a small amount of calcium solution directly to the blood in order to see if this hastened coagulation. As is well known, almost all investigators have found that the addition of calcium does not hasten clotting either in normal or hemophilic blood. Our first experiment agreed in this particular. In this instance we used 1/2 per cent. solution of $CaCl_2$ in normal salt solution. However, in subsequent tests the amount of calcium added was based on the calcium deficiency which had been found to exist in the blood. It will be seen that in the first test there was a deficiency of about 0.04 gm. of calcium per 1,000 parts. On the basis of this determination the deficiency was calculated for 10 drops of blood and a solution of $CaCl_2$ was made up so that the calcium in one drop about equalled this

TABLE II

	Control	Ca. 1	2	3	4
5 min.	–	–	–	+	+
6 min.	–	–	–	++	++
7 min.	–	–	+	++	++
8 min.	–	+	++	+++[1]	++
10 min.	+	++	++		+++
12 min.	++	+++	+++		
15 min.	+++[1]				

[1] Represents complete clotting.

deficiency. This is to say, one drop contained 0.04 mg. of calcium. This solution was then added to the blood.

The accompanying table (Table II) shows the result.

In the case of our patient, it will be seen that the calcium hastened coagulation and that the tube to which it was added clotted more quickly than the control tube which contained merely blood with normal salt solution to the same amount as the calcium solution. This experiment was repeated several times and upon each occasion the same result was obtained, that is to say the addition of calcium hastened coagulation. On the other hand, when this experiment was carried out upon normal blood, it was found that the calcium either did not hasten coagulation or indeed delayed it, as will be seen in Table III *a*. This was true likewise when calcium was added to the blood of a hemophiliac of the usual "family" type (table III *b*).

TABLE III *a*

Normal Blood

	Control	Ca. 2	3	4
4 min.	+	+	+	+
5 min.	++	++	++	+
6 min.	+++	+++	++	++
7 min.			++	++
9 min.			++	++
11 min.			++	++
13 min.			++	++
15 min.			+++	++
17 min.				++

TABLE III *b*

Typical Hemophilia

	Control	Ca. 1	2	3	4
4 min.	–	–	–	–	–
8 min.	–	–	–	–	–
10 min.	+	++	+	++	+
14 min.	++	++	++	++	++
18 min.	+++	++	++	++	++
22 min.		++	++	++	++
26 min.		++	+++	++	++
30 min.				+++	++

In addition to the tests of the calcium content of the blood and the tests on the relation of the addition of calcium to the clotting time of the blood, a metabolism experiment was carried out by Dr. Max Kahn, who has performed the chemical examinations in this study. In brief, the calcium was estimated during three periods, a fore period, a second period during which 2 gm. of $CaCl_2$ and 1 gm. of MgO were given, and a third period which corresponded to the first. This test was negative in every respect, excepting that there was found to be a negative balance of calcium during the fore period and a positive balance during the second period when $CaCl_2$ and MgO were given. This positive balance extended into the third period.

It may therefore be said that here is a case of hemophilia in which there is a definite deficiency of calcium. However, it has been found that this lack of calcium does not entirely explain the disturbance in blood coagulation, for even now when the calcium content is almost normal the tendency to bleeding is not entirely done away with and the coagulation of the blood has not reached the normal time. In other words there is another factor at play.

Discussion:

DR. LAMBERT: In regard to Dr. Hess's figures, I should not have thought that they indicated a very striking calcium deficiency in the blood. I recall that last year we made a number of analyses of dog's blood, using this same method of determining the calcium content. Our results showed a fairly marked variation in the amount of calcium in the blood of *normal* animals. Furthermore, it is known that blood may be diluted with many times (15–20) its volume of salt solution, thus reducing greatly the per cent. of calcium without disturbing to any extent the coagulation time. I was under the impression that a greater diminution in the calcium, than existed in Dr. Hess's case, would be necessary in order to affect appreciably the coagulation time.

DR. PAPPENHEIMER: Were there any other signs of deranged calcium metabolism such as in dentition? I should like to hear what the electrical excitability tests showed.

DR. HESS: In the first place concerning these tests: they are all low; one is so low that there cannot be any question concerning it and the others are also low. The last one is after giving 30 gr. of calcium lactate for three days. They are all lower than the controls. When the bleeding was most marked the calcium was particularly low. As regards the question of dilution: when you dilute the specimen, you also dilute the prothrombin. There is a relation between these two. You may dilute a specimen to 8 per cent.

and it clots as quickly. This is true of normal and of hemophilic blood, but you are diluting the prothrombin at the same time as the calcium.

The electric excitability tests were done by Dr. MacCallum. They seemed to be about normal, perhaps the responses were a little bit sluggish. I have noticed no disturbance in dentition. This child had some ichthyosis; whether there is any relation between calcium metabolism and ichthyosis I do not know.

THE EFFECT OF PENETRATING RAYS UPON EXPERIMENTAL TUBERCULOSIS

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I

In 1896 Lortet and Genoud[1] first mentioned the influence of X-Rays upon experimental tuberculosis. They used the simple X-Ray apparatus in vogue at that time and histological studies were not made. Rieder[2] in 1899 found that a certain inhibitory process was exerted upon local tuberculosis when the animals were exposed to X-Rays. Muhsam[3] inoculated guinea-pigs subcutaneously in the inguinal region and exposed this superficial situation to the X-Ray. At the point of inoculation he noted in the rayed animals limitation and encapsulation of the tuberculous process. The average length of life of control animals was 53 days and of experimental animals 67 days. Kupferle[4] later inoculated rabbits intravenously, produced tuberculosis and exposed these animals to penetrating rays; although the ray dosage appears to have been inadequate, he succeeded in producing a fibrosis of the tubercles in the lungs of the rayed animals.

Our experiments were undertaken with the view of determining whether the pathological changes in tuberculous animals were modified by the penetrating rays. Accurately weighed quantities of dried human tubercle bacilli, which had grown for 3½ weeks on Dorset's egg medium, were suspended in sterile salt solution and used for inoculation into guinea-pigs. The pigs varied in weight from 310 to 670 grams. The control and experimental animals in each case were approximately equal in weight.

Fourteen pigs were injected intravenously with 1/100 to 1/200 mg. of the dried tubercle bacilli in salt solution, while 13 were injected intraperitoneally with doses ranging from 1/100 to 1/10 mg. of the same culture. On the 12th day after inoculation guinea-pig No. 20 died, and autopsy revealed extensive tuberculous processes throughout all the organs and tubercle bacilli were demonstrated in the spleen. Of the remaining 26 animals, 7 controls died before the experiments were well under way and 3 experimental animals died after one exposure to the penetrating rays, necessitating the use, in some instances, of one control to 3 experimental animals. On the 32d day following inoculation exposures to the penetrating rays were made. The Gauss X-Ray apparatus was used and the rays applied to the dorsal, ventral and lateral surfaces over a period of 20 days, each animal receiving a dose of 8–10 X to each surface, in cross fire fashion, every 3d day. The focus distance was 20 cm., the penetration 10 B, and the length of exposure 6 minutes to each surface. The total dosage received ranged from 70 to 183 Kienbock units. The animals were bright and well and no external reaction was observed in any of them. The control animals lived 36, 38, 58, 60, 61 and 102 days respectively, the rayed animals lived 44, 52, 53, 57, 61, 79, 101, 101, 102 and 102 days respectively. The total length of life of 6 control animals was 362 days, a mean average of 60⅓ days. The total length of life of 10 experimental animals was 752 days, a mean average of 75⅕ days. Thus the experimental animals outlived the controls on an average of 15 days per animal.

The chart of duration of life of both sets of animals is given below:

The animals were autopsied in almost every case within 24 hours after death. Macroscopically they all showed tuberculous lesions in lungs, liver, spleen and lymph nodes.

The lungs of the controls upon microscopic section revealed intense congestion, hemorrhages into the alveoli, tuberculous pneumonia and numerous tubercles with central caseation. In the rayed animals on the other hand, we observed very few tubercles with cheesy centers, and many of the tubercles showed fibrous encapsulation.

Guinea pig Nos.		10	20	30	40	50	60	70	80	90	100	110	Days
29	.....	.....	.....	.....									36
27	+++++	+++++	+++++	-----	-----								44
34	.....	.....	.....	.....	.....	.....	.						61
23	-----	+++++	+++++	-----	-----	-							52
34	.....	.....	.....	.....	.....	.....	.						61
49	+++++	+++++	+++++	-----	-----	+++++	+++++	+++++	+++++	+++++	-----	--	101
52	.....	.....	.....	.....	.....	.....							58
30	-----	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	-----	--	101
47	.....	.....	.....	.....									38
50	+++++	+++++	+++++	+++++	+++++	+++++							57
40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...	102
22	+++++	+++++	+++++	+++++	+++++	-----	+++++	+++++	+				79
40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	...	102
24	+++++	+++++	+++++	-----	-----	---							53
19	.....	.....	.....	.....	.....	.....							60
41	+++++	+++++	-----	-----	-----	+++++	+++++	+++++	+++++	+++++	+++++	+++	102
19	.....	.....	.....	.....	.....	.....							60
53	+++++	+++++	-----	-----	-----	-----	-						61
19	.....	.....	.....	.....	.....	.....							60
45	+++++	+++++	-----	-----	-----	+++++	+++++	-----	+++++	+++++	+++++	+++	102

..... = control animals.
+++++ = treated animals.

In the liver of the control animal large areas of complete necrosis were seen, some of these areas involving an entire lobule. There were also numerous tubercles, originating in the portal spaces, presenting the picture of a progressive lesion with few epithelioid or giant cells. A few of the tubercles in the liver, notably those seen in sections from guinea pig No. 40, showed a certain amount of fibrosis. The rayed animals with the exception of No. 50 (which received 10 times the dose of the others) revealed fewer and smaller areas of necrosis; as a rule, the tubercles were miliary in character and frequently were surrounded by fibrous tissue; many appeared healed, and in some livers there were large atrophic areas about the portal spaces with connective tissue replacement.

In the spleen of some of the control animals very little normal tissue was visible; masses of necrotic and caseous tissue replaced the follicles. The sinuses were very wide and separated by little pulp tissue. With the exception of guinea pig No. 40 the tubercles in the spleen showed little fibrosis. In the rayed animals many tubercles in the spleen were completely fibrosed and few of them showed small caseous centers.

The lymph nodes of the control animals contained large areas of caseation and some small areas of fibrosis, while the nodes of the rayed animals contained a few tubercles with central caseation and many completely fibrosed tubercles.

From these experiments the following conclusions may be drawn:

1. Guinea-pigs in which experimental tuberculosis has been produced and which have subsequently been exposed to penetrating rays, live longer than their controls.

2. Caseation is less evident in the tuberculous lesions of the rayed animals, and fibrosis about the individual tubercles is more marked.

These conclusions may appear more significant when consideration is taken of the fact that radiation was instituted at an unfavorable stage of the disease, when the tuberculous process was widespread and had undergone caseation. Further experiments are being pursued to study the effect of radiation in the earlier stages of tuberculosis, and to determine the period at which these lesions are most favorably influenced. The details and protocols of the entire study are reserved for future publication.

1. LORTET AND GENOUD: Tuberculose Expérimentale Atténuée par la radiation Rontgen. *Gaz. des Hop.*, 1896, No. 78, p. 787.
2. RIEDER: *Münch. Med. Woch.*, 1899, No. 29, p. 950.
3. MUHSAM, R.: Versuche mit Rontgenstrahlen bei Experimenteller Tuberculose. *Dtsch. Ztschr. f. Chir.*, 1898, xlvii, 365.
4. KUPFERLE, L.: Experimentelle Untersuchungen uber die Rontgenbehandlung der Lungen Tuberkulose. *Strahlen Therapie*, 1914, No. 2, p. 500.

FAT METABOLISM IN TISSUE CULTURES

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The phenomenon of fat accumulation by cells growing in tissue cultures has been mentioned by nearly all of the workers in this field. The *cause* of the phenomenon and the *source* of the fat are questions, however, which have not been definitely settled.

Observations which will be discussed in detail elsewhere, have led us to conclude that the cause of the fat accumulation lies in a disturbance in the metabolism of the cells, resulting from (1) the relatively excessive quantity of fat which is offered the cells in pure plasma cultures, and (2) the absence of a circulatory mechanism in the culture preparations. We have concluded from the following experiments that the *source* of the fat is in the plasma medium; and not in the cells—a condition of fat accumulation, and not a fatty degeneration in any sense:

1. While cells growing in pure plasma become rapidly filled with numerous fat droplets, those growing in plasma diluted five to fifteen times with Ringer's solution accumulate far less fat, the quantity depending on the degree of dilution.

2. Cells growing in highly diluted plasma to which small quantities of glycerin and oleic acid have been added, accumulated more fat than the controls to which these fat components have not been added. This result has been interpreted as evidence in favor of an active fat synthesis.

3. The addition of such poisons as phosphorus and oil of pennyroyal to cultures in highly diluted plasma (low fat content) does not lead to an increased accumulation of fat.

CARCINOSARCOMA IN THE MOUSE

WM. H. WOGLOM, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director.)

Through the kindness of Dr. James G. Dwyer, our laboratory received last April a female Japanese waltzing mouse bearing two spontaneous tumors, one in the left axilla and one in the right groin, situations where mammary carcinoma is very likely to occur in the mouse (Fig. 1). Both tumors were extirpated and transplanted into three hundred and forty-eight white mice, in order to confirm the observation of Loeb and Tyzzer, that ordinary tame mice are naturally resistant to neoplasms of the Jap-

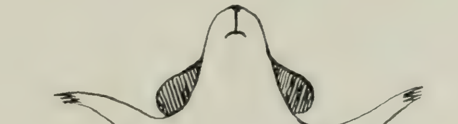

Fig. 1. Location and size of the two tumors in mouse 66/0. 6/7 natural size

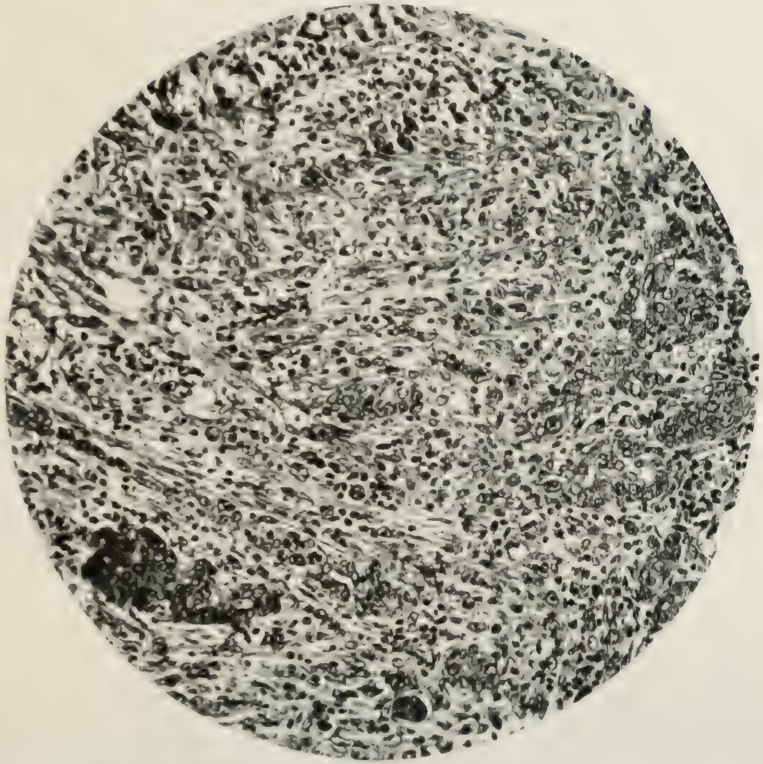

FIG. 2. Inguinal tumor. Carcinosarcoma. Hematoxylin and eosin. × 210.

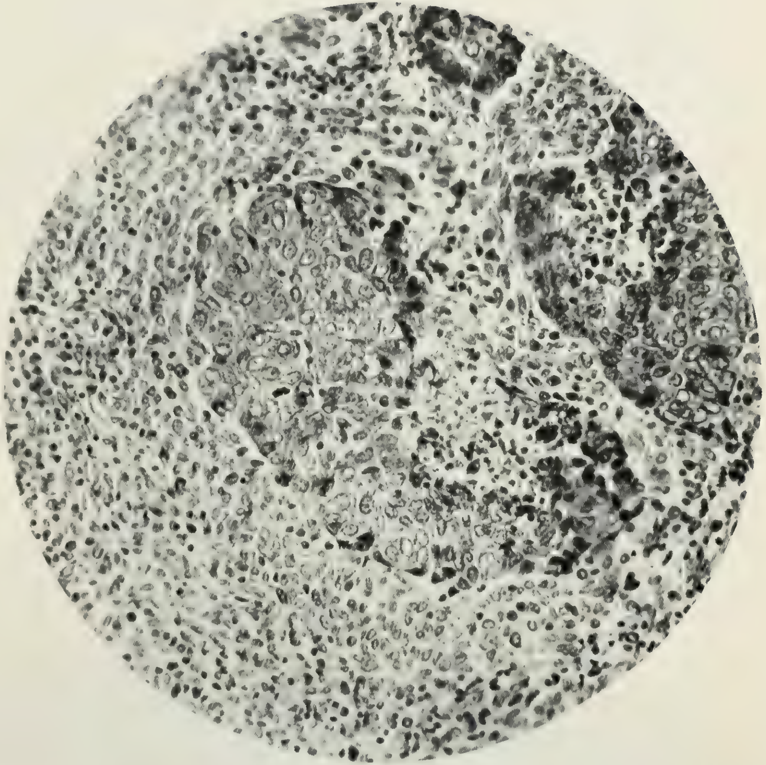

FIG. 3. Inguinal tumor. Carcinosarcoma. Hematoxylin and eosin. × 500.

anese waltzing mouse. Their findings were fully corroborated, since not one of all these animals developed a tumor. As the nature of the growths was not recognized at the time of operation, no attempt was made to procure Japanese waltzing mice for their propagation.

The tumor in the groin (Figs. 2 and 3) was found to be a carcinosarcoma, similar to those which have been already described by other investigators, while that in the axilla (Figs. 4 and 5) proved to be a pure sarcoma; still, its location in the mammary area and the fact that sarcoma of the mouse is relatively rare, while carcinoma is common, suggest that this tumor, like the former, was primarily a carcinoma. As a matter of fact, it did con-

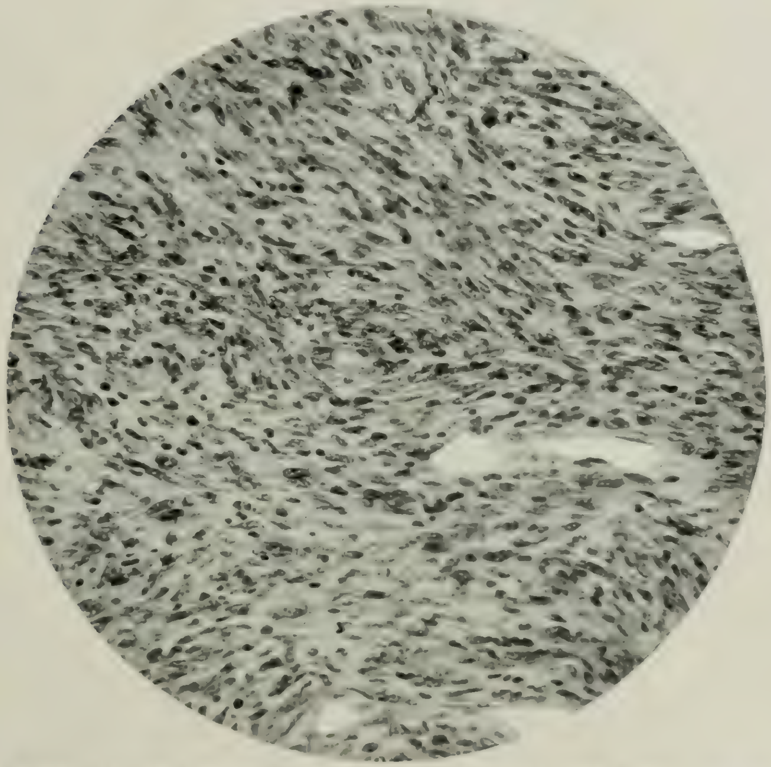

FIG. 4. Axillary tumor. Pure sarcoma. Hematoxylin and eosin × 250.

tain considerable epithelium, though the presence of this tissue gave no clue to the original nature of the growth, since it was undoubtedly normal lactating mamma that had been invaded by the sarcoma (Figs. 6, 7, and 8).

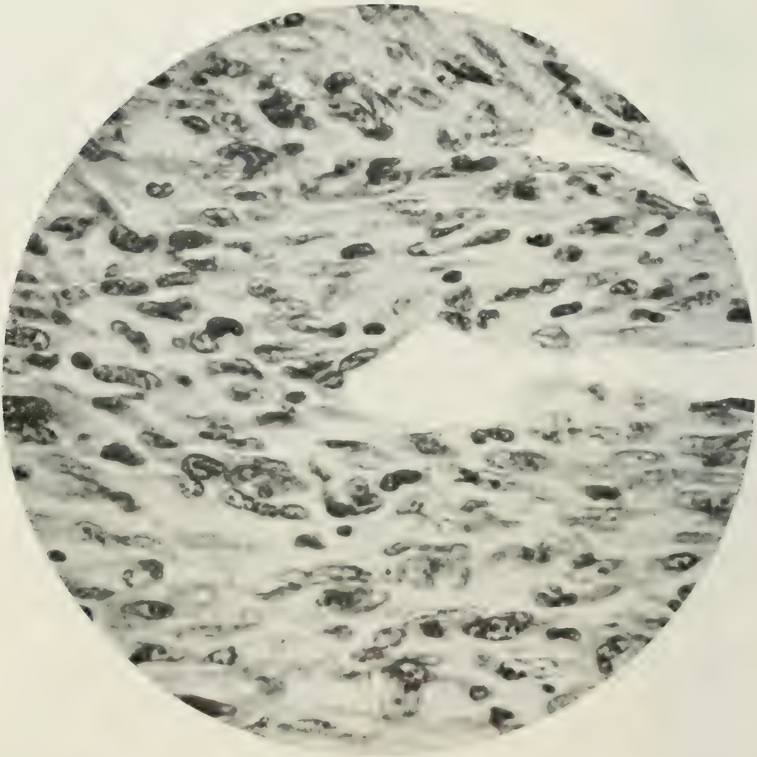

FIG. 5. Axillary tumor. Pure sarcoma. Hematoxylin and eosin. × 500.

In the case of the inguinal tumor, the newly developed sarcoma had not yet overgrown the carcinomatous constituent, while in the axillary growth it had succeeded in so doing. It is well known that, in transplantable carcinomata possessing the power to initiate sarcoma development in their stroma, the sarcomatous portion gradually gains the ascendancy over the carcinomatous, and there is no reason to suppose that this rule would not be valid in the case of spontaneous new growths.

Why the parenchyma of a few spontaneous and propagable carcinomata should be able to inaugurate sarcoma development in the connective tissue scaffolding, and in what manner it exerts this effect, are problems still unsolved. The power is ascribed by most investigators to the action of some stimulus, though what the nature of this may be even the boldest makes no attempt to define.

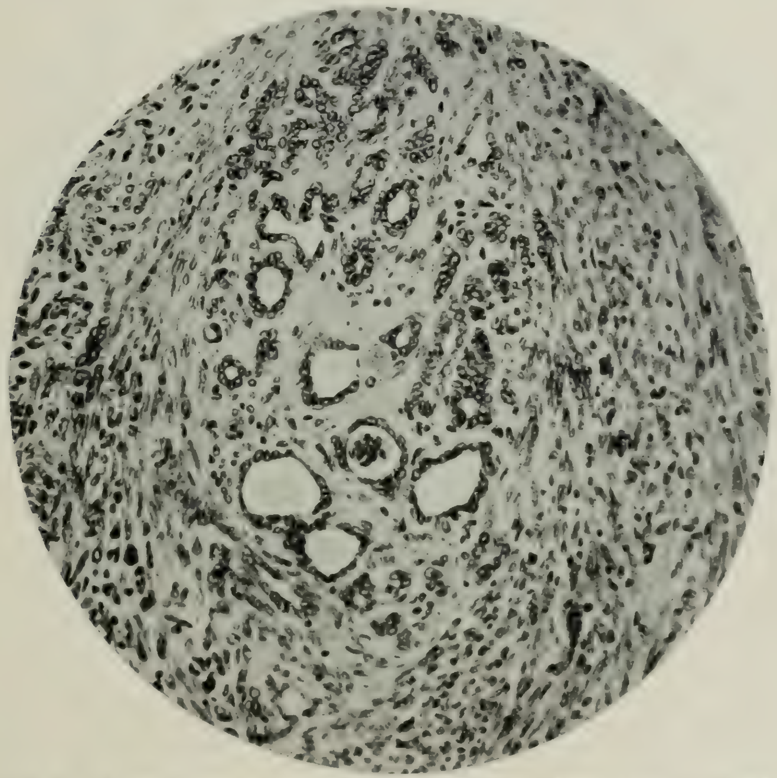

FIG. 6. Axillary tumor invading normal mamma. Hematoxylin and eosin. × 200.

The interest of the case now under discussion lies in the existence (or probable existence) of two carcinosarcomata in the same mouse. Ehrlich[1] has explained the onset of sarcomatous transformation in propagable carcinomata by assuming that the animal in which it takes place has some special predisposition toward connective tissue proliferation, as some human beings, for example, are apt to develop keloids at the site of a scar. To this hypothesis Russell[2] has objected that the Jensen mouse carcinoma, which has been propagated now for nearly fifteen years, has never yet given rise to sarcoma—has never yet, that is, happened to be inoculated into a "keloid" mouse, though a transplantable tumor able to cause this mutation will exert is effect in almost every animal, provided only that it be allowed to remain a sufficient length of time.

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[1] Ehrlich: *Ztschr. f. Krebsforsch.*, 1907, v. 64.

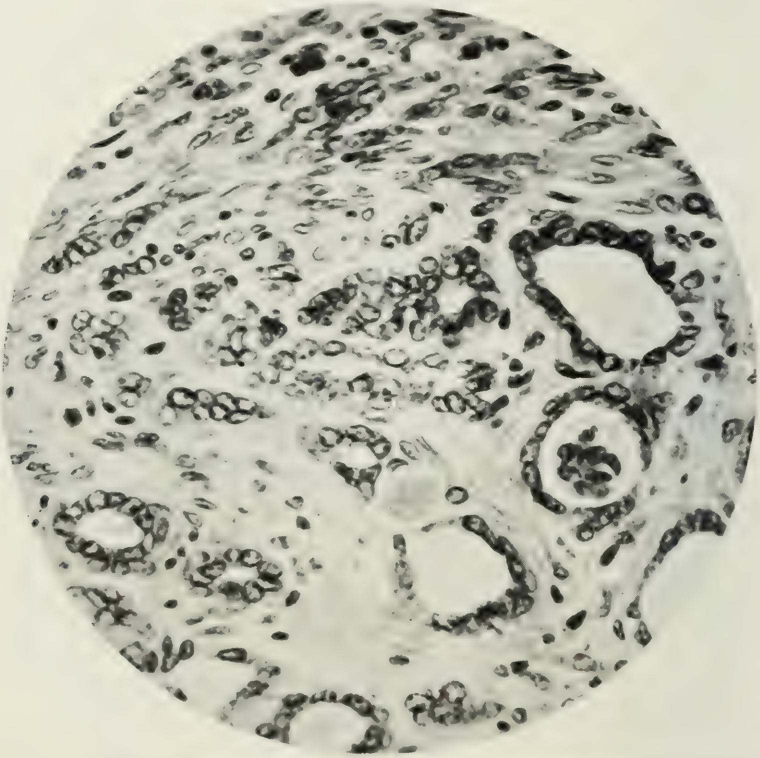

FIG. 7. Axillary tumor invading normal mamma. Hematoxylin and eosin. × 500.

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[2] Russell: *Jour. Path. and Bacteriol.*, 1910, xiv, 374.

If Russell's contention be correct, a mouse with sarcomatous transformation in two widely separated tumors is to be regarded, not as one in which the connective tissue is especially prone to undergo sarcomatous alteration, but as one whose mammary tissue, be it in one breast or another, possesses the power of inducing sarcoma development, once it has taken on malignant growth.

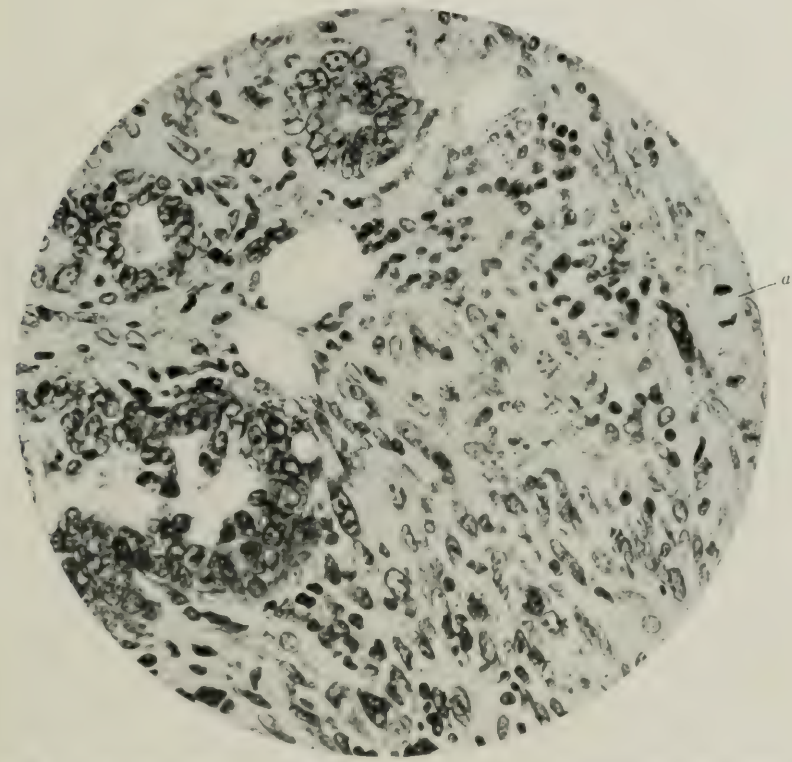

FIG. 8 Axillary tumor invading normal mamma. Hematoxylin and eosin. × 400. Note mitosis at *a*.

The observation supports certain others regarding the individuality of the tissues of any given mouse, suggesting, as it does, that although carcinomata of different mice vary among themselves, those of the same mouse may have characteristics identical, or nearly so.

IMMUNOLOGICAL RELATIONS OF A CHICKEN SARCOMA

WM. H. WOGLOM, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director.)

The employment of such terms as "sarcoma" and "tumor" throughout this paper is to be regarded rather as a concession to convenience than as indicating the possession of any definite idea regarding the material in question. The growth used in the following experiments is the first of that class to be reported by Dr. Peyton Rous, to whose courtesy our laboratory is indebted for the fowl from which the transplantations were made.

Because of the conflicting opinions regarding the nature of this tumor, it is a matter of some interest to ascertain whether chickens can be made resistant to it by previous treatment with embryonic fowl tissue, after the manner in which mice can be rendered refractory to mouse tumors with the embryonic material of their species.

A series of fowls were injected with hashed chicken embryo in amounts of from one to ten cubic centimeters, and inoculated with the sarcoma at periods varying from five to forty days later. Three weeks after the implantation of the tumor they were autopsied.

No reliable evidence of immunity was observed, as reference to the table will show. Since three treated chickens out of eight in the first group did not develop tumors, the experiment was repeated with double the amount of embryonic tissue; at the second trial the result was clean cut, none of the treated chickens being found resistant. The three other instances in which treated fowls did not develop tumors are probably chance occurrences, a view which is strengthened by the fact that Rous, also, has recorded quite recently his failure to immunize against this tumor.

On the other hand, the observation that some of the fowls engrafted with the sarcoma thirty-two and forty days after treatment, had smaller tumors than did those in the corresponding control groups, suggests that it may be possible, after all, to produce immunity to this growth, though it is evident that the development of the refractory state would take much longer than the ten days requisite in the mouse. It is intended to continue the experiment as soon as fertile eggs can be obtained in sufficient number.

TABLE I

Interval Between Treatment and Tumor Inoculation	Dose of Embryo Emulsion	Number of Chickens	Tumors	No Tumors
5 days	5.0 c.c.	8 treated, 10 controls[1]	10	0
			5	3
5 days	10.0 c.c.	6 treated, 6 controls	6	0
			6	0
10 days	1.0 c.c.	17 treated, 15 controls	15	0
			16	1
12 days	5.0 c.c.	10 treated, 11 controls	11	0
			10	0
14 days	2.0 c.c.	9 treated, 5 controls	5	0
			9	0
14 days	4.0 c.c.	5 treated, 2 controls	2	0
			5	0
25 days	5.0 c.c.	11 treated, 10 controls[1]	10	0
			10	1
28 days	2.0 c.c.	3 treated, 3 controls	3	0
			3	0
32 days	5.0 c.c.	3 treated, 5 controls	5	0
			2[2]	1
40 days	5.0 c.c.	7 treated, 11 controls	11	0
			7[2]	0

Even though it be finally proved impossible to immunize chickens against this tumor, the observation would be no unanswerable argument against its sarcomatous nature, for, in the first place, it is not known that the immunological reactions characteristic of the mouse have their counterpart in the chicken, and, secondly, mouse tumors are occasionally found against which no resistance can be produced. It could be interpreted, at best, only in conjunction with such facts as have been already ascertained, or as may be discovered in the future.

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[1] Same controls used for both experiments.

[2] More small tumors than among controls.

Discussion.

Dr. Ewing: Considering the great frequency with which polyhedral cells are combined with spindle cells in human carcinomas and the clear evidence that these spindle cells are derived from the polyhedral cells, I have been surprised at the readiness of experimental pathologists to accept the conclusion that epithelial tumor processes are capable of exciting sarcomatous changes in the stroma. One of the first principles of tumor diagnosis is that considerable areas of spindle cells may occur in atypical carcinomas. This fact is not sufficiently recognized. I have examined many reports of the so-called carcino-sarcomatous growths and have been unable to satisfy myself that the spindle cell tissue was sarcomatous and not derived from epithelial tissue. In every case that I have seen outside the group of true mixed tumors the spindle cell areas have apparently been derived from polyhedral cells. I am not willing to express an opinion as to the significance of the tumors in lower animals; that should be left to those who are extremely familiar with the subject, but I have been fortunate enough to see two examples of so-called transformation of the stroma into sarcoma. In these two instances the specimens were submitted to me for proof of the sarcomatous change in the stroma. I did not however, reach this conclusion. I think the epithelium is transformed into spindle cells. I would not express a positive opinion without elaborate investigation, but I do not think that the conclusions drawn in this field have adequate support.

Dr. Rous: Last year Dr. Jones and I found three carcino-sarcomata among 135 mice with spontaneous tumors. The opportunities to study the condition were unusually good. The growths occurred in animals used in experiments on the influence of diet upon the development of recurrences and of disseminated fragments of spontaneous tumors. The mice were operated upon with incomplete removal of the tumor and the implantation of bits of it elsewhere. By the time these bits had begun to grow, or the tumor had recurred, a section of the primary growth was available for diagnosis.

Of the three carcino-sarcomata one seemed peculiar in the gross. It was a disc-shaped tumor in the mammary gland, giving retraction of the nipple. The second carcino-sarcoma was especially interesting. The primary growth was a pure carcinoma, the recurrence a mixed tumor and the growing grafts from the primary tumor consisted of pure sarcomatous tissue, although the material used for implantation had, so far as investigated, no such a constituent. All three of the carcino-sarcomata grew rapidly and were uninfluenced by diet.

Attempts were made to transplant the tumors; one of them grew rapidly for about ten days, but then underwent retrogression and was lost. Before our next attempt at transplantation we happened to read an article by Haaland, in which he mentions the general tendency of mouse sarcomata to retrogress after rapid initial growth. Accordingly, we re-transplanted the next carcino-sarcoma during the period of initial growth and were successful in propagating the tumor in this way. The growth regularly retrogresses after a few weeks. In the first generation it became a pure sarcoma.

Dr. Woglom says that in his opinion the occurrence of two sarcomatous tumors in one mouse suggests a special peculiarity of the tissues of this mouse. But is there not an alternative explanation? Multiple tumors are fairly frequent in the mammary glands of the mouse. We have noticed as many as five in one animal. Russell has pointed out that probably only one of such growths is primary, the others representing disseminations of it. In favor of this we have the fact that such multiple tumors are as a rule of identical histological structure. The mere fact that the tumors are widely separated is not good evidence against a metastatic origin for some of them.

We have made two attempts to immunize chickens against the chicken sarcoma with embryonal tissue, but both were unsuccessful. However, our experiments differed from Dr. Woglom's in that the tumor was implanted within a few weeks after the inoculation of the embryonal tissue. It seems very probable that the immunity induced by embryonal tissue is dependent on the absorption of the artificial teratoma produced by its growth. In the chicken such teratomata persist for a long time.

Dr. Woglom: In reply to Dr. Ewing, I can only reiterate my belief that those growths in the mouse which have been described as mixed tumors are actually carcinosarcomata. There occurs an entirely separate type of tumor, a pure carcinoma, composed partly of spindle-shaped epithelium, but here, as was pointed out, the transition between round and spindle-shaped cells is gradual, while in the carcinosarcomata it is abrupt.

Regarding the question of Dr. Rous, the possibility of one of the two tumors being a metastasis from the other was considered and disregarded.

Replying to Dr. Pappenheimer, a basement membrane was demonstrable in tumor 66.

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DR. ALWIN M. PAPPENHEIMER, *President*

THE PATHOLOGY OF BUNDLE BRANCH LESIONS OF THE HEART

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and

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The subject of the pathological anatomy of heart block is undergoing refinements. Until recently the only form of dissociation recognized in the heart was that between auricles and ventricles. Cases, however, were occasionally found not to be due to transverse lesions of the *A–V* bundle, as, for instance, the large number of instances which occurred during intoxications of one or another sort. Recently during galvanometric examinations of the heart, unusual curves were obtained, which permitted the suggestion that, even though no injury to the main stem of the conduction system was present, a defect in conduction from the auricles to a single ventricle existed, due possibly to a lesion of the bundle branch to this ventricle or to one of its subdivisions. A clinical observation (1) suggested this possibility, and a subsequent experiment (2) confirmed it.

That bundle branch lesions might cause the sort of asynchronism between the ventricles which v. Leyden and his followers had in mind as determining the gallop rhythm, led to the first experiments of cutting the left branch by Barker and Hirschfelder (3). In their disappointment in not producing heart block in their successful experiment, they concluded "that the His bundle plays little if any rôle in the coordination of the two ventricles." The conclusion is erroneous, in the light of later investigations, and would certainly not have been drawn if electrocardiograms could have been taken.

The clinical observation which suggested this form of lesion was made by Eppinger and Stoerk (1). They report five cases in which they made the diagnosis and found lesions to correspond in the right branch of the *A–V* bundle. The electrocardiographic evidence depends on the width of the *R*-wave. They lay stress on the inversion of the *T* wave, but do not insist that it always occurs; they are, in fact, not very clear about it. Actually, their criteria for making this diagnosis are not convincingly stated. Hering and Kahn objected to the criteria which were used, whereupon Rothberger and Winterberg (4) later repeated their experiments and came to the extraordinary conclusion that in order to make the diagnosis of a unilateral bundle branch lesion leads I and III must be taken, but the two leads must yield curves having deflections directed in similar fashion! This demand is based on their experiments. If the diagnosis is to rest on this basis, no human cases so far described have corresponded to it.

In the original experiments which Eppinger and Rothberger (2) carried out, they cut one branch in some, and the other branch in other animals. They obtained curves corresponding to contraction in the ventricle to which the branch was left intact. The cutting of a single branch failed to produce auriculo-ventricular block, just as in the experiments of Barker and Hirschfelder, but when the two branches were cut in succession, complete block occurred, and the form of the curve differed little from the control. It is a matter of interest to note that Eppinger believed gallop rhythm to be present in his clinical and in this experimental experience. Similar cases have since been studied by Cohn and Lewis (5), Lewis (6), Mathewson (7) and Carter (8).

Lewis (9) calls curves which show the abnormalities "aberrant." The beats are associated with conduction changes referable to the junctional tissues. Carter (8) defines them more closely and shows that, aside from conduction changes, the initial ventricular group *Q–R–S* "exceeds 0.1 second, and as a rule constitutes more than one third of the entire complex." The waves are large, of bizarre forms, and the final deflection *T* is directed opposite to the initial ventricular wave.

The four cases of which histological studies were made, and which are now reported, are included in Lewis's (10) studies of hypertrophied hearts, and are to be found there, numbered 42, 44, 73, and 91. They all gave signs of bundle branch defects electrocardiographically, and are to be reported in detail later. A detailed report of the anatomy follows.

No. 42.—This heart, as well as the other three to be described, was received, already divided into portions appropriate for weighing. The portion with which this investigation is concerned, that is to say, the lower portion of the interauricular, together with the greater part of the interventricular septum, appeared as a single piece, covered only in part by endocardium. The extent of the endocardial covering measured approximately 3 cm. below the attachment of the aortic valves. This line represents the base of a triangle, the apex of which appeared near the apex of the ventricle. It is clear that as one approached the apex, the endocardium present narrowed. This dissection was unfortunate, because the lower levels of the branches of the *A–V* system spread out laterally, the nearer the apex is approached. The endocardium of the septum on the right side fared no better. It covered an area below the attachment of the tricuspid valve measuring 4 cm. wide, and this piece also gradually tapers toward the apex. The septum was cut so that it did not extend backward far enough to include the opening of the coronary sinus. It is apparent that with the small amount of endocardium left in place, an insufficient examination of the *A–V* system in continuity was possible. The piece embedded embraced the greater portion of the septum, still covered by endocardium.

The *A–V*[1] node in this heart was a well-developed but, relatively speaking, a small structure. Neither it nor the main stem which springs from it presented an abnormality. The latter was composed of fewer fibers than usual, and gave the impression of being attenuated. At its forward end, before it divided into its two branches, a number of small thin-walled blood sinuses appeared. After the division of the main stem, both right and left branches were rather slim bundles of muscle. The right branch was soon lost; at least, its dimensions were so small that tracing its progress became impossible.

Tracing the onward course of the left branch was also a matter of much difficulty. That part of the endocardium which covered it was very much thickened by the growth of new connective tissue. In this situation it is normally, as Nagayo (11) has shown, a very complicated membrane. Hyperplasia and diffuse infiltration of connective tissue destroyed the possibility of identifying its layers. With the destruction of the normal arrangement of the endocardium and the lack of distinctiveness in the normal histology of the conducting muscle, the recognition of the latter was impossible. The growth of new connective tissue was not confined to the endocardium, but also involved the tissues directly underneath, including the left branch of the *A–V* bundle.

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[1] The technique of examination has been described in a paper by Cohn, Holmes and Lewis, *Heart,* 1911, II, 241–248.

Under the endocardium it showed no tendency to destroy the muscle fibers, but just to burrow among them and to separate them.

The fibrosis extended into the substance of the septum. The right third showed practically normal relations between muscle and connective tissue. But the two thirds to the left replaced much of the original muscle tissue. When sections stained with picric acid and acid fuchsin were viewed without lenses, this portion appeared almost red as contrasted with the yellow of the right third.

Summary.—The *A–V* node and the main stem were normal. The main stem just before its division contained dilated blood sinus. There was extensive increase of connective tissue in the endocardium and subendocardial tissue, as well as extensive growth of it within the substance itself of the ventricular septum.

No. 44.—This heart was prepared in the same way as the preceding one. The septum of the auricles was so cut that it excluded the coronary sinus, but the *A–V* node was left in continuity with the septal piece, as the sections showed. The *A–V* node was well developed. The main stem passed forward through the membranous septum and divided normally into the two branches. Both branches were followed without difficulty throughout the extent of the block of tissue. The only abnormality detected was in the left branch. This consisted in a distinct diminution in the number of fibrils of the muscle elements. Their absence occurred in the centers rather than in the periphery of the cross sections and gave the fibers the appearance of the familiar Purkinje cells. The heart muscle fibers were practically normal. A few small areas in which there were sclerotic patches were found in the septum, but these were, relatively speaking, insignificant.

Summary.—The *A–V* node, main stem and branches showed no lesion interfering with their continuity. The fibers of the left branch showed an absence of fibrillæ at their centers. There were slight sclerotic patches in the interventricular septum.

No. 73.—In this case the septum of the heart was cut so as to include the opening of the coronary sinus. The dissection of the heart necessitated the same sacrifice of the endocardium and subendocardial structures near the apex which has been mentioned in speaking of the other hearts. The piece embedded embraced a block of tissue including the greater part of the septum.

The *A–V* node, main stem and its division into right and left branches presented no abnormalities. Both branches were followed through the series without difficulty, and presented no lesion. The muscle of the interventricular septum, however, showed a very marked increase of connective tissue. This appeared both as thick bands but also as finer strands passing between individual muscle fibers. The muscle was in many places distinctly atrophic and occasionally showed sarcolemmas all but empty of fibrils. Other fibers were small and shrunken, the nuclei poorly stained. In these areas there were many thin-walled vessels having rather large lumina,—dilated, new-formed blood sinuses. There was no evidence that this lesion represented an acute inflammation, for there was no infiltration with leucocytes or lymphocytes. The degenerated area was quite extensive and in cross section measured in places 6.0 by 1.5 mm. At some levels it extended toward the lumen of the left ventricle and lay almost under the endocardium, in close proximity to the left branch of the *A–V* bundle. No embolus or thrombus was seen. There was no fragmentation of the heart muscle.

Summary.—*A–V* node, main stem and branches were practically normal. In the interventricular septum there was a rather large area showing extensive degeneration. There was no evidence of recent acute inflammation.

No. 91.—This heart was cut in the same way as the others; the septal piece did not include the opening of the coronary sinus. The auriculo-nodal junction and the *A–V* node were normal. The main stem contained a larger amount of dense strands of connective tissue than is normal, but this did not interfere with the continuity of the conducting system. It also contained many small, thin-walled blood vessels. There was no other evidence of inflammation. The main stem divided into right and left branches in the center of the membranous septum and not at its forward extremity as is usual. In following the branches of the *A-V* bundle further, the small, thin-walled vessels followed the right branch exclusively. The walls of these were thick and dense, due to connective tissue increase. This lesion characterized the right branch as far as it was followed. The fibers of the left branch showed an absence of fibrils about their center similar to that described in heart No. 44. This appearance was confined to the left branch. The fibers of the rest of the heart muscle did not show the vacuoles described. There was fragmentation of the muscle. The transverse striations were well preserved. Scattered through the substance of the muscle there were numerous large areas of dense connective tissue, which has replaced the muscular fibers.

Summary.—The *A–V* node was normal. The main stem contained more than the usual amount of connective tissue, and at its division thin-walled blood sinuses. The latter were present in the right branch as far as it was followed. The fibers of the left branch showed atrophy of fibrils at the centers. There was moderate sclerosis of the myocardium.

In these four hearts, then, no lesion interfering with the continuity of the main branch of the conducting bundle or of its branches was seen. In the one heart, No. 42, in which the branches could not be followed, the failure to do so was due to an increase in connective tissue at the site of its passage, so that wanting a distinctive histology, its continuity was lost. In the three other hearts a similar difficulty was not encountered. Neither was an acute inflammation of the conducting system found in any. One of the hearts showed the presence of thin-walled blood sinuses encroaching on the area of the right branch of the bundle, and in two hearts there was a lesion of the left branch, causing a disappearance of the more mesially placed fibrils in the muscle fiber, giving these the appearance of Purkinje cells. Similar fibers have been described in the auricle in connection with accounts of the structure of the sinus node and have been mistaken there for Purkinje cells. In an examination of many human hearts, a similar vacuolization of the conducting fibers has escaped detection, so that it must be regarded as extremely doubtful that the fibers described are normal.

The four hearts had all of them very decided lesions of the myocardium in the form of connective tissue growth of one or another form, replacing the proper muscle tissue of the heart. Sometimes the fibrous change consisted in small dense patches, at others of a more diffuse, infiltrating (interstitial) character, and sometimes it was associated with extensive degenerative changes in the muscle fibers going on without acute inflammation.

CONCLUSION

The failure to find lesions in these hearts to correspond with electrocardiographic disturbances may be due to the impossibility of complete examination on account of the defects in the specimens. Even if eventually, after complete and satisfactory examinations have been made, no constant lesion shall have been found in bundle branches, the disturbance in conduction may still confidently be assigned as the cause of electrocardiographic change. The reason for so doing rests on the evidence gained from main stem lesions. Auriculo-ventricular block admittedly takes place without a lesion of the main stem. The *A-V* bundle is certainly at fault, for it alone is responsible for carrying impulses. Yet in certain cases where incoordination exists, no demonstrable lesion has been found. It may, therefore, be the more readily believed that failure in a branch or a group of branches to conduct is not due to a change in an anatomical sense. No structural defect need therefore be found. The difficulty is one which cannot be solved in the domain of pathological anatomy. The fact that anatomical destruction is unnecessary is seen in the fact that bundle branch blocks disappear just as those of the main stem do (12). It is impossible to attempt to estimate the influence of the alterations found in the ventricular septum on the passage of impulses over the conduction system, or on the contraction of the ventricles and consequently also on the electrocardiogram.

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Discussion

Dr. Oppenheimer: We are running through two hearts now where the electrocardiograph in one indicates a right bundle lesion, and in the other where a left bundle lesion is indicated. Macroscopically, we have found nothing; the microscopic studies have not yet been made.

Dr. Pappenheimer: What is the nature of lesions which have been found in the previously reported cases?

Dr. Cohn: Simply hemorrhages and fatty degenerations. Mönckeberg has included these in his special pathology.

Dr. Pappenheimer: Is there degeneration of the conduction fibres?

Dr. Cohn: There are two kinds of lesions in the tissue. First, there is fatty infiltration of the muscles; whether this has functional significance is not known; second, the fibres of the conducting muscle atrophy at the centers of the fibres; the nuclei are isolated; the fibres lie in a peripheral relation to the tube of the muscle fibre; they look like Purkinje fibres and have been mistaken for them.

Dr. MacCallum: How is the curve related to the lesion?

Dr. Cohn: It is impossible, in the present state of our knowledge to say; the first tall peak probably represents the passage of the impulse through the body of the ventricle; in that case it is not an expression of contraction of muscles; the second wave is associated with contraction and represents an electrical phase common to all muscles in contraction.

Dr. MacCallum: If Dr. Cohn interprets the change of direction in the first curve as due to a different direction of the impulse, can he apply a similar interpretation to the small curve?

Dr. Cohn: These points are being studied in a more detailed way. Einthoven has worked out a method by use of three leads, for arriving at a manifest potential difference which makes it possible to calculate the direction of the action current at any instant of time; changes in the direction of the electrocardiographic waves will acquire much more significance in the light of these investigations and this knowledge it is hoped will answer questions that are not answerable at present.

EXPERIMENTAL CARDIAC ENLARGEMENT AND ACCOMPANYING ELECTROCARDIOGRAPHIC CHANGES

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In 1912 Einthoven pointed out that from the size and direction of the waves in the three leads of an electrocardiogram it is possible to calculate mathematically the angle between the electric axis of the heart and the axis of the body. It has further been observed that in patients with left or right ventricular hypertrophy the electrocardiograms are indicative of a turning of the electric axis of the heart to the left or right. In patients suffering from acute or chronic diseases, qualitative and quantitative changes in the waves are observed that cannot be accounted for satisfactorily, so we endeavored to see whether definite changes in rabbits' hearts were accompanied by definite quantitative or qualitative changes in the electrocardiograms. As the rabbits were observed over long periods of six or seven months care was taken that the electrocardiographic records should be strictly comparable. X-ray photographs were taken from time to time, when indicated, that the appearance of gross changes could be noted during life. These were in all cases taken with the tube at a distance of 2½ feet vertically over the lower end of the sternum. After death the hearts were divided and weighed according to the method of Müller and histological examinations made.

The methods used to obtain the changes consisted in the intravenous injections of (1) adrenalin, (2) spartein sulphate and adrenalin, (3) diphtheria toxin, (4) suspension of living streptococci. In all cases where enlargement of the heart to one side or the other was obtained, definite accompanying electrocardiographic changes were noted. These enlargements were most marked in the cases where injections of spartein and adrenalin were employed by the method recommended by Fleischer and Loeb.

Lantern slides illustrating the changes in the X-ray picture and in the electrocardiograms of three rabbits were shown, illustrating enlargements to left, to right and in both directions. Slides showing tables of the changes throughout the observations on these and on four other rabbits were shown and the result of the histological and gravimetric examination after death briefly described. The technique employed was not sufficient to allow of accurate estimation of the changes in the *P* and *T* waves, but it was shown that enlargements of the heart to one side or the other were accompanied by definite quantitative changes in the Q., R. and S. waves, an enlargement to the left being accompanied by diminution in the upwardly directed waves and increase in the size of the downwardly directed waves, and reverse changes accompanying right-sided enlargement.

Discussion:

Dr. Pappenheimer: I would like to ask Dr. Fraser whether similar alterations in the electrocardiogram have been observed in human beings, and if so, in what type of cases. Are they found in simple hypertrophy, such as that associated with renal disease?

Dr. Fraser: I think the only cases that have been described are those showing high pressure while under observation. The heart shows definite increase of the ventricle to the left side with an increase of the electrocardiographic deviations downwards and decrease of the deviations upwards. This is not described in dilatation cases.

Dr. Pappenheimer: We had recently at the Presbyterian Hospital a case of extensive infarction of the left ventricle following thrombosis of the coronary artery. Did Dr. Fraser find any characteristic electrocardiographic changes in this case?

Dr. Fraser: Yes: a left-sided enlargement was shown.

Dr. MacCallum: Do you regard these alterations in the electrocardiogram as evidence of change in the nature of the work of the heart? Or is it merely change in the origin of the impulse? I should think you have to deal with a number of factors. There is probably an increased amount of work going on, certainly when you are giving adrenalin. There must be very considerable change in the heart muscle itself and modification of the work of the heart by changes in the aorta and other vessels.

Dr. Fraser: Einthoven and Waller, and Lewis, in very recent work, have come to the conclusion that the *Q. R. S.* waves are an indication of stimulation; their direction and size may be considered as indicating relation of the angle of the axis of the heart to the angle of the body. It is not known what the *P.* and *T.* waves mean. One can only say that the electrical axis of the heart changes when the heart hypertrophies; there are no indications of changes in the work.

Dr. Cohn: It is important to point out at this time that the nature of the technique employed for correlation of the electrocardiogram with the mechanism of the heart must be changed, if we are to make progress. The literature deals with examinations of the heart taken at a single instant, rarely repeated, and rarely followed for a period of study synchronous with the length of the disease. These experiments of Dr. Fraser's begin to fill a want, in supplying a series of observations extending throughout the whole period of disease. On the basis of these it will be possible to correlate all the changes which are found and to discover their relation.

ABDOMINAL PREGNANCY WITH REPORT OF A CASE

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HISTORICAL REVIEW

Among the earlier writers upon the subject of ectopic gestation the belief was general that there was a type of primary abdominal pregnancy. Recent contributors to the subject are quite unanimous in the opinion that there are probably no authenticated cases on record of primary abdominal pregnancy, most of the so-called cases being due to a secondary rupture of a broad ligament pregnancy.

During the sixteenth century the difference was recognized between primary and secondary rupture; but there seems to be no accurate record as to who first described abdominal pregnancy. Some sixteenth century writers mention cases in which the foetus was found in the abdomen. The earliest case of abdominal section for ectopic gestation was that of Primerose in October, 1594. The next report was by Felix Platerus in 1597. There were no other similar reports for a century. The next records are those of Calvo, in France, in 1714, and Bard, in America, in 1764.

Until 1824 but three types of ectopic gestation were recognized, namely (1) ovarian, (2) tubal, and (3) abdominal. In that year Breschet added the interstitial variety; and in 1837 Dezeimeris added the subperitoneo-pelvic variety, which we now speak of as "broad ligament pregnancy."

In 1876 Parry proposed a very elaborate classification including ten different varieties. Lawson Tait in 1888 substituted a much simpler classification, marking an epoch in the study of this condition. His classification, which in its essential features, is generally accepted at the present time, is as follows:

I. Ovarian.—Possible, but not proven.
II. Tubal (free portion).
    1. Primary rupture. (Always before the fourteenth week.)
        (*a*) Intraperitoneal.
        (*b*) Extraperitoneal.
    2. Secondary rupture.
        (*a*) Intraperitoneal.
        (*b*) Extraperitoneal.

Primary intraperitoneal rupture is in most cases fatal, unless immediate operation is performed; whereas if the primary rupture is extraperitoneal, or between the layers of the broad ligament, it may proceed to full term in that situation, or rupture into the peritoneal cavity. In the latter event the placenta is usually attached to the walls of the ruptured cyst.

Tait asserted that in normal conditions of the pelvic viscera conception always occurs in the uterus; whereas, Webster, Sutton, Martin and Veit on the other hand maintained that conception may take place in the normal tube. Concerning the abdominal variety Tait did not accept the possibility of an ovum dropping into the peritoneal cavity, because the powers of digestion of the peritoneum are so extraordinary that an ovum, if fertilized, could have no chance of development. All abdominal gestations are, in his opinion, either ruptures from the tube or broad ligament. Bland-Sutton expresses an identical opinion, and Webster in his monograph on ectopic pregnancy, published in 1895, says: "The long-held belief that an early complete ovum may escape into the cavity of the abdomen and develop on the peritoneum is a pure supposition unsupported by any evidence whatever. On the other hand, all our knowledge tends to show the exceeding great improbability of this view."

Many causes have been assigned for the development of the ovum outside of the uterus. The following list given by Webster would seem to cover most of the conditions which have been assigned as possible causes.

1. Mechanical.
    - (*a*) Peritoneal bands constricting the tube.
    - (*b*) Polypi in lumen.
    - (*c*) Tumors of the surrounding parts.
    - (*d*) Abnormal foldings of the tubal mucous membrane.
    - (*e*) Diverticula from the lumen.
    - (*f*) Displacements and hernia of the uterus and its appendages.
2. Interference with peristaltic action.
    - (*a*) Adhesions between the tube and neighboring parts.
    - (*b*) Thickening of the walls by inflammation.
3. Destruction of the ciliary action of the epithelium of the tube from inflammatory changes (endosalpingitis).

Parry and his predecessors, Astruc, Rambotham and Brandelocque, and others, attributed many of these cases to moral and mental influences, such as fright, strong passion, etc. Astruc considered the fear of detection among those practicing illicit intercourse, or emotional disturbance experienced at or just after intercourse as contributory causes of extra-uterine pregnancies. These are now universally rejected as having no physiologic basis.

The following observation made by Parry is quoted verbatim: "Women may have a previous inaptitude for conception. The interval between marriage and the first conception is frequently long. If the woman has borne children, a period of sterility frequently precedes the extra-uterine pregnancy."

The literature contains many unusual cases. Among five hundred cases reported by Parry he classified fifty as being of the abdominal variety. Many of the records of the cases reported by him are now so inaccessible that the evidence presented cannot be weighed. Those which have been examined, however, by subsequent observers have been considered as probably cases of tubal or ligamentous pregnancies.

In 1869 LeCluyse reported an unusual case in which a Cæsarian section was performed at the time of the first pregnancy. The second child died in the eighth month. Four days later a laparotomy was performed and the fœtus removed from the abdomen. The mother died on the tenth day, and at autopsy an oblong opening was found on the anterior surface of the uterus, from which the conclusion was drawn that the ovum had been fertilized in the uterus and proceeded through this opening into the abdominal cavity. These deductions have been severely criticized by those who have reviewed the report since that time, and it has been considered as a probable tubal or tubo-ligamentous pregnancy.

Koeberle reports a case in which the body and part of the neck of the uterus were removed. The woman later became pregnant, went to term, and died undelivered. On necropsy a fistula through the cicatrix of the neck was discovered. One does not know from the report how much of the uterus was taken away. Enough may have been left to communicate with the tube, or the tube may have become adherent to the uterine stump, the cavities communicating, so that gestation may have started in the uterus. It is also possible that it was tubal. Webster states that it is an entirely unwarranted assumption to say that it had passed into the peritoneal cavity.

Velpeau asserted that he had seen two cases in which the fœtus was found in the abdomen and had no connection with the ovary, tube or uterus. Matecki reported a similar case.

Turnbull in 1792 reported a case in which no placenta was found, the anterior wall of the cyst, or vesicle, measuring 1/10 of an inch. The fœtus was nourished by the meso-colon. The left tube was obstructed by a pelvic tumor. This was probably a case of ruptured tubal or ligamentous pregnancy.

The case of T. R. Jessup, reported by Tait, has probably received more attention than any of the others. There was evidence of a primary rupture at the tenth week. At the eighth month there were unmistakable symptoms of a secondary rupture. The mother was twenty-six years of age. Her last pregnancy preceding the one reported occurred in March, 1869. In January, 1875, her menstruation ceased. In March of the same year she became violently ill with pain in the right side, retention of urine, high pulse, etc. For two months she was confined to bed. In May she felt fœtal movements, and a tumor was found well down on the right side. An operation was performed in August by Tait, and a child was found free in the abdominal cavity. It was in a kneeling position with the head upwards and the buttocks presenting toward the umbilicus of the mother. The placenta was attached to the structures at the brim of the pelvis, to a portion of the abdominal wall, and to the large intestines. The omentum was draped over the shoulders of the child like a cape.

Bandl reports a case in *Die Krankheiten der Tuben* in which the mother arrived at full term, the fœtus being extra-uterine. She refused operation and later succumbed from peritonitis. At autopsy the child was removed alive, but unmistakable evidence of rupture of a broad ligament cyst was present.

Champney's case, operated in June, 1886, in which the child was found free in the abdomen with no membranes, was proven at subsequent autopsy to have been a rupture from the right broad ligament. The placenta was above the pubis. The left tube was normal; the right tube could not be made out.

Taylor in 1890 reported a case somewhat similar, but expressed the opinion at the time that it was a tubal or tubo-ovarian pregnancy.

In a case reported by Sutton he was able to isolate the lower part of the right broad ligament forming the sac, the placenta occupying the pelvis and part of the posterior wall of the uterus beneath the peritoneum. The corresponding tube and ovary were not detected. Sutton reported this as a case of so-called abdominal pregnancy.

In 1903 C. P. Noble exhibited a specimen of abdominal pregnancy which was removed at the end of twenty-one months. The left side of the pelvis was roofed by adhesions between the broad ligament and the sigmoid. The amniotic fluid was absorbed and the fœtus was adherent to the sac. There were unmistakable symptoms of a primary rupture at the end of two months. At seven months the mother became ill with chills and fever, and was treated as typhoid at the time. This was unquestionably the period at which the secondary rupture occurred.

Guiterrez described a case of abdominal pregnancy in 1904 in a foreign magazine to which the writer has not been able to have access. The opinion has been expressed by Adami that this is the only case that contains evidence which is at all convincing that gestation occurred primarily in the abdomen. The latest case reported is that of Ellis McDonald in November, 1911.

There has been a considerable difference of opinion as to whether the decidua is always formed in the uterus or in the extra-uterine sac. Virchow was of the opinion that it was always formed in the uterus. For eighteen years Dr. Robert Lee asserted in a dogmatic fashion that the decidua always occurred in the sac. Some observers have reported it in both the uterus and the sac. The universal opinion at the present time is that the decidua always forms in the uterus. Parry's conclusions regarding this are: First, in all cases the decidua forms in the uterus; second, it is rarely retained until the completion of term; but is thrown off during the false labor, or if the pregnancy goes to term it is expelled in small fragments without being observed; third, the absence of the decidua is no proof that it has not been formed.

The placental attachment is a point of great importance in the decision as to the place in which gestation has occurred. It has been found to be attached to the omentum, stomach, mesentery, colon, bladder, posterior abdominal wall, and to the pelvic viscera.

The termination of the chorionic villi do not enter the membrane to which the placenta is attached; but spread out upon it. The coats of the villi are usually single. The placenta is usually thinner than the normal; but it has been reported in one case to be three inches thick. The uterus is, however, almost always adherent to the chorion. In ventral gestation, whether primary or secondary, the sac is composed of the ovular envelopes of the fœtus alone, or conjoined with an adventitious membrane formed as the result of irritation. Some cases have been reported in which the child has been found uncovered by any membrane, or surrounded only by an adventitious cyst. The former observation was made by Carl Braun, and the latter by Bandl. Lufkin and von Neugebauer report six cases in which the fœtus was free among the intestines. The amniotic fluid is usually small in amount, and may be completely absorbed. The child may occupy any position in the abdomen. The fœtus is usually smaller than the normal, and may be deformed; but monsters are seldom met with. In cases going beyond term a cartilaginous change may take place in the fœtus. It may become calcified, forming a lithopœdion. Mummification may occur, or the fœtus may be converted into adipocere. There is, however, little evidence of this latter change. Suppuration may intervene, producing symptoms of peritonitis, demanding operation.

CASE REPORT

The patient from whom the specimen to be demonstrated was removed died in the Tuberculosis Division of the Metropolitan Hospital on November 6, 1914, the necropsy being performed on the following day. During her stay in the hospital no diagnosis of pregnancy was made, there being no evidence to suggest this condition. Her abdomen was not enlarged. Although she was requested by her friends to inform the physicians in attendance regarding her previous symptoms of pregnancy she refused to do this, but requested that in case she did not recover she would like to have an autopsy performed in order that her abdominal condition might be observed.

The *necropsy* showed extensive tuberculous lesions of both lungs with multiple cavities. The heart showed brown atrophy of the musculature. The liver was in a condition of congestion with moderate degeneration of the liver cells. The other organs showed no significant lesions.

Upon opening the abdomen a tumor mass was encountered, extending from the pelvis to four fingers' breadth above the umbilicus. It was situated between the ascending, transverse and descending colon, to which it was attached by a fibrous membrane. The omentum was firmly attached to the anterior surface of the tumor from which it could not be separated. On incising the membrane, it was found that the anterior portion of the tumor consisted of the placenta, which measured 18 cm. in its superior-inferior diameter, and 13 cm. in its lateral diameter. At its center it was 1.5 cm. in thickness, and near the edges 3 mm. in thickness, gradually tapering off into the membranes of the sac. The cord was attached eccentrically between the center and the right border of the placenta. The length of the cord was 27 cm. The fœtus lay in the sac with the head down in the left occipito-posterior position. The face showed some deformities from pressure. The amniotic fluid was very small in amount, and was of a brownish turbid color. The fœtus was covered with vernix caseosa. The skin resembled that of a normal child at birth. The head was covered with long black hair. The nails had grown beyond the tips of the fingers and the toes. The inferior portion of the sac was adherent to the fundus of the uterus, but no connection with tube or broad ligament could be demonstrated. The posterior wall of the sac was formed by a thin membrane. The tubes, ovaries and broad ligaments on each side could be demonstrated. The left tube measured 9 cm. the right, 7 cm. The right tube was slightly tortuous near its distal extremity, but the left was quite normal. The weight of the child was 1,200 grams.

The uterus was normal in size, measuring 7.5 by 4 cm. The thickness of the wall of the body was 7 mm. The endometrium of the uterus appeared perfectly normal, no decidual changes being noted. The cervix was small and hard.

Upon inquiry from friends some information was obtained regarding the *previous history* of the patient before her admission to the hospital. It was learned that she visited other hospitals and clinics during her pregnancy, and from investigation of these records, together with the information obtained from friends, a fragmentary history has been obtained.

In January, 1913, she was delivered of her first child by a Cæsarean section performed because of eclampsia. Examination of the records of the hospital in which this operation was performed revealed nothing unusual.

The fruitful coitus is said to have occurred on October 10, 1913. The last menstruation occurred on September 11, 1913. During the early months of pregnancy the patient is said to have complained of abdominal pains every afternoon, which were so severe as to cause her to go to bed. Early in March, 1914, fœtal movements were felt.

In the last week of April, 1914, she is said to have had very severe pains in the abdomen, more on the left than on the right side, starting in the illiac region and extending up the left side of the abdomen. There was no hemorrhage at this time. She was told by a physician whom she consulted that these pains were labor pains.

During the early part of May she went to a hospital in Manhattan several times; but was told on each visit that it was too early for her delivery. After one of these visits, Friday, May 8, while on her way home, she fell in the street and was returned to the hospital in an ambulance. At this time the abdominal pain was so severe that " she could not stand up nor sit down." No hemorrhage occurred, however. On the following day she was sent home from the hospital. During Saturday and Sunday the pains continued, and she was confined to her bed. On the following Monday, May 11, 1914, she was again taken to the same hospital where she remained three or four days. The following physical examination was taken from the hospital records:

"Last menstruation, Sept. 11, 1913; Para 2. Expected confinement, June, 1914. Diagnosis, pregnancy.

"Menstrual life began at 14 years of age; regular; duration 4 days. Previous pregnancy, Cæsarean section January, 1913.

"*Physical Examination.*—Heart, normal. Lungs show signs of bronchitis. Breasts: Glandular tissue fair in amount; nipple prominent; secretion of colostrum. Vagina: Varicosities; no indications of venereal disease. Perineum intact.

"Abdomen: Striæ present. Ballottement present. Fœtus: Vertex (?) presentation; fœtal heart not audible. Uterus: No contractions seen; size large; form pyriform. Cervix: Position central and forward. No softening. External os: Normal. Internal os: Not open. Lacerations: None. Pelvis: Right side under developed. Left side roomy. Doubtful if fully developed fœtus can be delivered.

"Thought to be in the sixth month of pregnancy."

A few days after her discharge from the hospital she passed some blood while at the toilet. The only description that could be obtained of the blood was that it was thick and black. After this her menstruation was resumed, and it occurred regularly. During the month of May she ceased to feel life. She said that she sometimes felt movements in her abdomen; but there were no kicks, and it did not feel as it had before. At this time she became very worried.

In July or August she went to a clinic (definite information as to what clinic has not been obtained) where she was examined and told that she would be confined in a few days, and she took a room near the clinic for two weeks. At this time she began to develop a cough.

In September she consulted a physician, who, after examination, told her that he did not know whether she was pregnant or not. During this same month she went to a hospital where she was told that she would have to wait longer for her baby. It was during this month that she was transferred from a hospital in Manhattan to the Metropolitan Hospital.

In June her abdomen began to reduce in size. This diminution in size progressed rapidly until the evidence of an abdominal tumor entirely disappeared.

SUMMARY

From the anatomic evidence there is nothing to show that this was a case of a ruptured broad ligament or tubal pregnancy. The tubes, ovaries and broad ligaments were normal, showing no evidence of a previous rupture. The uterus was normal in size and contained no decidua. Although the tumor was adherent over a small area to the fundus of the uterus the connection was apparently an incidental one. If the condition of primary abdominal or intraperitoneal pregnancy is a physiological possibility this case seems to supply the evidence necessary to prove such a condition.

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FŒTAL REMAINS IN THE ABDOMINAL CAVITY

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It is a remarkable coincidence that a few days after the examination of the case just presented we should come across a similar case of abdominal pregnancy, incidentally a broad ligament pregnancy. The death of the fœtus in such cases as these often results in danger to the mother from peritonitis, but, exceptionally, conditions arise in which the remains of the fœtus are got rid of by the body passages. In this case it was so.

Few cases of abdominal pregnancy have been presented at this Society, although I find, upon going into the records, as far back as 1870, that pathologists, obstetricians and gynecologists came here to present unusual tubal cases and abnormalities of the genito-urinary tract of all kinds. So far as I am aware no similar case has been put upon the records of the Society. This patient was a woman, 51 years of age. The menopause occurred at 45 years. The history obtained from the husband was that the last pregnancy occurred at 44 years. After the menopause she commenced to suffer from bleeding from the rectum and saw many doctors without avail. For two years she had been gradually becoming more and more anæmic. She went to one of the maternity hospitals where they found a mass in the region of the caput coli. The X-ray gave a dull shadow. The woman was then transferred to Bellevue Hospital where she was observed for a few weeks and discharged. She was then taken to the City Hospital in a moribund condition and very cachectic. A diagnosis of carcinoma of the coecum was made. After four or five days she died.

At *autopsy* a peculiar condition was found. The abdominal viscera were matted together forming a very large mass in the region of the cecum. This was removed en masse, together with the adherent sigmoid and rectum. On examination of the cecum it was found to contain parts of a fully developed fœtus, giving the measurements of a normal child at term. The parietal and temporal bones and the zygomatic processes were all in the cecum and a mass of pultaceous material. The appendix was not found. The adherent rectum entered directly into the cecum, also the sigmoid flexure, so there were four openings into the cecum where the full-grown fœtus was found. This case had a very definite history. The husband said she had been passing with the feces objects that looked like bones.

Such cases as this are extremely rare. They have been described by Playfair and I saw a case he had described. In one case a full-grown fœtus remained in the abdominal cavity thirty years. One woman carried a fœtus fifty-two years. In these cases the fœtuses remained in the peritoneal cavity as innocuous bodies. The general tendency, however, after the death of the fœtus is expulsion through the abdominal wall. Bland Sutton has also described these cases. Sometimes the fœtus ruptures into the bladder or vagina. There are very few cases on record where the fœtus had gone on to full term. It is unusual for two such cases to be found in one hospital in one week.

THE RELATIONSHIP OF THE LIVER TO THE CHOLESTERIN METABOLISM[1]

M. A. ROTHSCHILD, M.D.

The rôle of the liver in cholesterin metabolism has given rise to much speculation and theory. Naunyn years ago expressed the view that the epithelium of the gall-bladder and finer bile ducts secrete lipoids, especially cholesterin. This theory has again been advanced by Chauffard and his pupils, who maintain that a real synthesis of cholesterin occurs in the finer bile ducts. Aschoff and his pupils in their work on the etiology of cholelithiasis claim that when Sudan-staining substances are found in the epithelium of the gall-bladder, it is a resorptive phenomenon due to the oversaturation of the bile with cholesterin.

However, several fundamental facts stand out as of paramount importance, namely; bile normally contains only free cholesterin and not cholesterin esters, whereas the blood contains both free cholesterin and cholesterin esters. Furthermore, in physiological and pathological states no anisotropic substances are found in the liver. By chemical examinations, the liver may be shown to contain relatively small quantities of cholesterin, with small but varying percentages of cholesterin combined with fatty acids.

Those working in the field of cholesterin metabolism are divided into two camps. The first, the French school, maintains that a real synthesis of cholesterin occurs in the body, this synthesis occurring in the cortex of the adrenal glands, the corpus luteum and the finer bile ducts. The second, or German school, regards the adrenal cortex as only a storehouse for lipoids.

In a previous communication I showed, first, that in a rabbit with no adrenals there was a tremendous increase of cholesterin in the blood, also the more adrenal tissue remaining, the smaller the percentage rise of cholesterin in the blood; and second, that in a unilateral adrenalectomized rabbit if the cholesterin content of the remaining adrenal falls too low the animal dies with the characteristic symptoms of adrenal insufficiency, exactly as the animals with no adrenals. For these reasons, one can exclude any synthesis of cholesterin in the adrenal glands and must conclude that lipoids in general are essential for the proper correlation of cortex and medulla and possibly for the entire sympathetic nervous system.

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[1] This work was carried on at the pathological laboratory, University of Freiburg, under the direction of Professor Aschoff. I wish to take this opportunity to thank Professor Aschoff, Professor Autenrieth and Dr. Landau for their kindly assistance.

Previous experimental work has developed several interesting facts. First, Dorée, Fraser, Gardner, and others have shown that the fully developed chick contains only as much cholesterin as the egg. Second, Bacmeister has shown that in dogs there is a retention of lipoids in pregnancy. In man the same phenomenon has been demonstrated by numerous authors. Previously, Kusumato had shown that 70 per cent. of the ingested cholesterin fails to appear in the feces. Grigaut, the writer, and others have shown that it is possible to cause a hypercholesterinemia by feeding cholesterin or cholesterin-rich foods. From these facts alone it would appear that the organism is born with a definite quantity of cholesterin and that this quantity is maintained by the food intake.

In order to throw more light on the subject, I examined the food intake, the blood, and the bile of a series of animals—herbivora, carnivora and omnivora. There is a striking parallelism between the amount of cholesterin in the food intake, the amount present in the blood and the amount excreted through the bile. In the herbivora, taking the rabbit as an example, the food is poor in cholesterin, containing a metameric product or phylo-cholesterin. In the blood of a rabbit small quantities of cholesterin, varying from 30 mg. to 80 mg. per 100 c.c. of blood, are present. The bile contains only traces of cholesterin, amounting to 5-15 mg. per 100 c.c. Some observers, Weltmann, for example, have denied the presence of cholesterin in rabbit's bile. However, the error lies in the method used. By feeding a rabbit cholesterin it is possible to increase the cholesterin content of the rabbit's blood threefold without causing any marked increase in the bile. The cholesterin metabolism of these animals is so sluggish that a hypercholesterinemia develops before any increase of cholesterin in the bile is observed. This fact explains the artificial production of arteriosclerosis by Anitschkow by feeding cholesterin.

The diet of carnivora, such as dogs or cats, is rich in cholesterin. In these animals one finds large and varying quantities of cholesterin in the blood and corresponding amounts in the bile. By feeding cholesterin to dogs it is not possible to cause a high grade of cholesterinemia, even though very large quantities are used since the excess that is absorbed is promptly excreted in the bile. A cat fed fifty-nine days on cholesterin showed 109 mg. of cholesterin per 100 c.c. of blood, and 180 mg. of cholesterin per 100 c.c. of bile, while a rabbit fed the same period of time on smaller amounts showed 364 mg. per 100 c.c. of blood, and 160 mg. per 100 c.c. of bile. It has not been possible, because of the active excretion of cholesterin in the bile of carnivora, to produce artificial arteriosclerosis by cholesterin feeding.

In pigs, as a class of omnivora whose diet corresponds more closely to that of man, we find varying, but large quantities, of cholesterin in the blood, and in a series of examinations of the bile of pigs amounts varying from 60 to 180 mg. of cholesterin per 100 c.c. of bile were found.

An analysis of these facts, when taken in conjunction with the previously stated evidence, seems to show conclusively that our supply of cholesterin is maintained by our food intake and that a cholesterin equilibrium is maintained by the excretion of the excess through the bile. The bile, however, contains only free cholesterin and not cholesterin esters, while the blood is rich in cholesterin esters. The question therefore arises as to whether certain cells of the liver are not concerned in the de-esterization of cholesterin before excretion. General catabolic processes liberate free cholesterin and cholesterin esters to the blood stream.

In order to throw some light on this subject the livers of bilateral and unilateral adrenalectomized animals were examined morphologically and chemically. Chemically, all showed an increase in the cholesterin content corresponding to the amount present in the bile. In the early stages of hypercholesterinemia there is an increase in the cholesterin content of the liver in rabbits before any increase manifests itself in the bile. However, with the further development of a higher grade of hypercholesterinemia large quantities of cholesterin are found in the bile With the stoppage of the feeding of cholesterin, increased quantities continue to be excreted in the bile, and as a result a gradual decrease of the cholesterin content of the liver is observed. It appears therefore that on feeding cholesterin there is an increase of the cholesterin content of the liver followed secondarily by an increased excretion in the bile, and that on stopping the administration of cholesterin the decrease is observed in the liver before it is manifested in the bile.

Morphologically, one finds in the livers of the bilateral adrenalectomized animals anisotropic substances, occupying a radial position about the central vein, and extending out between the columns of liver cells. These substances stain with nile blue and Sudan, proving them to be a mixture of cholesterin esters, fatty acids and free cholesterin. These anisotropic substances occasionally show the so-called Kreutz figures. These are soluble in ether and alcohol, disappear on heating, reappear on cooling, thus giving the typical reactions of cholesterin esters. Stained with hematoxylin and eosin one finds that these anisotropic substances are only present in the Kupfer cells, the endothelial cells intermediary between the capillaries and liver cells. These cells show evidence of proliferation and are increased in size. Their ability to take up colloidal substances from the blood stream is not purely confined to cholesterin as they absorb iron pigment, bacteria, carmine, etc. In the liver cells or epithelium of the bile ducts no anisotropic substances are present. It is possible that these specialized endothelial cells, the Kupfer cells, may have a function of de-esterization, occupying as they do intermediary positions between the capillaries and liver cells. The finding of fatty acids mixed with these anisotropic cholesterin esters in these cells also speaks for their possession of a de-esterizing function.

The livers of the *bilateral* adrenalectomized animals were also examined, in view of the contention of Naunyn and Chauffard that a synthesis of cholesterin occurs in the epithelium of the bile ducts and gall-bladder. In order to produce the most favorable experimental conditions for studying the supposed function of the bile ducts, I used male rabbits thereby obviating any corpus luteum effects, and removed another of the theoretical organs of cholesterin manufacture, the adrenal glands. In these animals the complete absence of anisotropic substances in the epithelium of the bile ducts or gall-bladder as well as the minute quantity of Sudan-staining substances in these tissues makes the theory of Chauffard, at the least, improbable.

From these facts one can exclude any synthesis of cholesterin in the body. We are supplied at birth with lipoids and this supply is maintained by our food intake. Excesses of cholesterin are excreted through the bile as free cholesterin. The cholesterin metabolism of carnivora and omnivora is so labile that normally any increase in the food intake is immediately excreted through the bile.

In view of these facts the common bile ducts of several dogs were tied. This caused a retention hypercholesterinemia, the increase in the blood being at the expense of free cholesterin and not cholesterin esters. These animals were then fed large quantities of free cholesterin, as much as 5 gm. a day. No greater hypercholesterinemia was produced than that explainable by the usual retention following the tying of the common bile duct. The cholesterin as free cholesterin in the absence of bile is thus not absorbable from the intestinal tract. Landau has examined the fluid obtained from the thoracic duct after a meal rich in cholesterin and found it to be rich in cholesterin esters and minute quantities of free cholesterin. It would thus appear as if bile is essential for the esterization of free cholesterin. As cholesterin esters, the cholesterin is absorbed by the lymphatics and by way of the thoracic duct enters the blood stream for distribution to all the tissues in the body. Eventually, in the course of normal catabolic processes, it is returned to the liver, where it is probably de-esterized in the endothelial cells and excreted through the bile again as free cholesterin.

The next problem presenting itself is whether this free cholesterin in the bile is not in turn re-esterized in the intestinal canal and possibly reabsorbed. To investigate this point a series of rabbits were starved from one to nine days and a daily examination of their blood was made. An examination was also made of the bile and organs at the time of death or at the time of killing of the animals. Depending upon the length of the period of starvation, a hypercholesterinemia develops. The animals starved nine days showed the greatest increase in blood cholesterin. The bile, as well as the liver and adrenals, was rich in cholesterin. Eight cubic centimeters of bile stained fluid was found in the duodenum which also contained large quantities of cholesterin. From these experiments it appears that the free cholesterin in the bile is re-esterized in the duodenum, reabsorbed and reaches the blood stream by way of the lymphatics. This must be a protective mechanism on the part of the organism to prevent a depletion of the body lipoids.

An interesting clinical fact develops from these starvation experiments. Normally, in the gall-bladder of rabbits 3–5 c.c. of bile, very poor in cholesterin, is present. In these starved animals, however, 10–15 c.c. of bile were found and the bile was extremely rich in cholesterin. Aschoff has previously pointed out the frequent development of gall stones in those individuals who have lost weight very quickly through the so-called reducing cures. The finding of increased quantities of bile with a marked increased content of cholesterin in the above animals supports this observation of Aschoff.

Summary.—1. The cholesterin content of the blood and bile of herbivora, carnivora and omnivora is dependent upon the amount of cholesterin ingested in the food. In carnivora and omnivora any increase of cholesterin in the diet manifests itself by an increased excretion of cholesterin in the bile and not by hypercholesterinemia, while in herbivora an increased intake causes a hypercholesterinemia and only later an increase of cholesterin in the bile.

2. The bile contains only free cholesterin and not cholesterin esters, while the blood contains a considerable percentage of chol-

esterin esters. Normally, the liver contains small quantities of cholesterin and no anisotropic cholesterin esters are present.

3. In rabbits made hypercholesterinemic by feeding cholesterin there is an increase of cholesterin in the liver followed by an increase in the bile. By stopping the cholesterin-rich diet the hypercholesterinemia decreases until normal quantities are present in the blood, the liver gradually loses its cholesterin corresponding to an increased excretion through the bile.

4. In bilateral adrenalectomized rabbits there develops a hypercholesterinemia, followed by an increase in cholesterin content of the liver, and later by an increased excretion through the bile. Morphologically, anisotropic substances, giving the typical reactions of cholesterin esters, are found in the endothelial cells of the liver (the Kupfer cells). No anisotropic substances are present in the liver cells or epithelium of the bile ducts. These endothelial cells, part of the "endothelialer Stoffwechselapparat" of Aschoff and Landau, may be regarded as intermediate organs of cholesterin metabolism and may possibly be concerned with the de-esterization of cholesterin.

5. In the cholesterin rich liver of the hypeercholesterinemic rabbits which excrete large quantities of cholesterin in the bile, one finds no lipoids in the epithelium of the finer bile dhcts and very minute quantities in the epithelium of the gall-bladder. A synthesis of cholesterin in these cells is not probable.

6. In dogs, whose bile ducts have beeen tied, free cholesterin is not absorbed. Bile is thus essential for the esterization of cholesterin and as cholesterin esters it is absorbed by the lymphatics and thence passed into the blood stream.

7. In starved rabbits, there develops a hypercholesterinemia, with increased quantities of cholesterin in the liver, adrenals and bile. The quantity of bile is also increased, and cholesterin-rich, bile-stained fluid is present in the duodenum. There is then a resorption of the free cholesterin from the duodenum. This is a protective mechanism of the body designed to prevent any depletion of lipoids.

CONCLUSIONS

1. The complete cholesterin metabolism is probably as follows: There is no synthesis of cholesterin in the body. Our supply is maintained by our food intake. Free cholesterin is esterized in the intestinal canal, absorbed by the lymphatics, delivered to the blood stream and distributed to the body cells. With the breaking down of cells as in general catabolic processes, it is again freed to the blood stream, taken to the liver where the endothelial cells possibly produce a de-esterization, an intermediary part, excreted with the bile as free cholesterin, and again partially re-esterized and reabsorbed from the intestinal tract by means of the lymphatics.

2. The liver, therefore, and not the adrenals, must be considered as the organ of the body which normally controls the cholesterin metabolism.

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Discussion:

Mr. Mueller: Concerning the excretion of free cholesterol only, in the bile, and the suggestion of a possible de-esterizing function of the Kupfer cells, there are one or two further facts to be considered. Kusumoto and others have advanced the idea that the biliary cholesterol comes from the broken-down red blood cells. This is substantiated by the fact that injections of certain hæmolytic substances, such as p-toluyldiamin, is followed by an increased excretion of cholesterol in the bile of dogs having a permanent biliary fistula. Furthermore, analyses by Kanders show that the red cells of most animals contain practically no cholesterol esters, but simply free cholesterol. In view of these things, a de-esterizing function of the liver would not be necessary to account for the presence of only free cholesterol in the bile. On the other hand, it has been shown by Schultz and by Gardner that the livers of certain animals, notably the horse and the cat, when autolyzed for some time, show an increase of free cholesterol at the expense of cholesterol esters, and this result is accentuated by the admixture of whole blood, or red blood cells, but not by serum, from the same animal. Although this has not been found true of the liver of other animals, it is evidently a chemical verification of the histological findings reported in the previous paper.

In the light of the recent work of Lipschütz there may be some reasonable doubt as to the complete conservation of cholesterol in the body. He has shown by the absorption spectra of certain color reactions that there is found in the body a substance indentical with oxycholesterol, which may readily be prepared synthetically by the oxidation of cholesterol. This is found in larger quantities in the blood entering the liver than in that leaving it. In the liver, however, there is an abundance of unsaponifiable material other than cholesterol, giving other color reactions which are again given by further synthetic oxidation products of cholesterol, to which definite formulæ have not been assigned. And finally, the bile acids on oxidation give identical spectra. Hence it is concluded that some cholesterol is oxidized in the tissues to oxycholesterol, the latter is brought to the liver and further changed into other substances, of which the cholic acids are the most important.

Advocates of the theory that cholesterol is not synthetisized in the body bring forward the evidence that the total cholesterol in eggs does not increase on hatching. Mendel, working from a different point of view, found a decided decrease during the period of incubation. A careful analysis of the work of other observers shows in every instance a small but definite decrease when the averages for a number of determinations are taken, which they ascribe to experimental error. It is difficult to understand, however, why experimental error should invariably be in the direction of decrease, never of increase. May it not be true that the formation of oxycholesterol and the bile acids may account satisfactorily for this decrease?

Dr. Rothschild: In my paper this evening I purposely omitted all controversial points as they were immaterial to the general principles I have attempted to evolve. Lifschütz has claimed the existence of oxycholesterin which he calls "activated cholesterin." The existence of such a substance is still controversial. The existence or non-existence of the substance is immaterial as far as the general principles advanced in this paper. The question of a special enzyme in the liver for the de-esterization of cholesterin is equally controversial. The main point is that certain cells of the liver have this function, and whether or not this function is performed by an enzyme is relatively unimportant.

A far more important point arises, and that is whether cholesterin contributes to the formation of bile acids. All evidence is in favor of the view that taurin has its origin in the breaking down of the protein molecules. Of the origin of the nitrogen-free bile acids we have no definite knowledge

However, present evidence also points to the protein molecule as its origin. Nevertheless, it may be possible that the fat metabolism contributes slightly to this formation.

The objections raised to the work of Mendel on the cholesterin content of eggs before and after incubation are threefold: First, the present methods for cholesterin determination are only comparative and not absolutely accurate; second, the observations of Mendel are so few that the finding of a general decrease in the cholesterin content of eggs after incubation may be purely coincidental; and third, it is a well known phenomenon that heat destroys the cholesterin molecule.

THE MECHANISM OF ANAPHYLACTIC SHOCK

RICHARD WEIL, M.D.

The problem of anaphylaxis is now almost ten years old. During this period it has been the object of a greater amount of investigation than any other branch of immunity. There is a widespread impression, probably owing to the multiplicity of publications and the confusion in terminology, that the subject itself is very difficult and complex. Such, however, is not the case. Most of the difficulties have been met and overcome, and at the present time the subject rests on a clear and simple foundation.

In the present paper I shall limit myself to the consideration of one phase of the subject, namely, the mechanism of anaphylactic shock. The explanation of this phenomenon confronted the very earliest observers, but in a form entirely distinct from that which now interests us. They were occupied chiefly with two problems. The first of these concerns the question of the identity, or non-identity, of the sensitizing and of the intoxicating substance. As the outcome of a long period of discussion, with which are chiefly associated the names of Gay and Southard, and of Besredka on the one side, and of Rosenau and Anderson on the other, it was definitely established that these two functions are performed by exactly the same substance. The second question involved the problem of the reaction of the animal to the sensitizing substance, as to whether or not this reaction depended upon the production of antibodies of the general nature of precipitins and hemolysins. This problem, too, owing largely to the work of Rosenau and Anderson, of Doerr, and Friedberger, is finally settled in the sense that the production of specific antibodies to the sensitizing substance is the essential factor in the development of the hypersensitive condition. As a result of this earlier work, then, it is definitely established that the introduction of a foreign protein by a parenteral route results in the formation of specific antibodies. Upon the second, or intoxicating, injection of the same foreign protein there is a reaction between the antigen and antibody which results in the production of anaphylactic shock.

More recent investigation has concerned itself especially with the *site* of this reaction. This problem was first clearly defined by Otto in 1907. He discovered that a sensitized or immunized animal contained in its blood substances which were capable of conferring the same anaphylactic condition when introduced into a normal animal. But he went further than this; he showed that passive sensitization had a distinct latent period. In other words, the normal animal developed the hypersensitive condition only several hours after the introduction of immune serum. From this fact Otto argued that the presence of antibody in the serum could not in itself confer hypersensitiveness, but that the cells themselves had first to anchor this antibody. Hence he concluded that anaphylactic shock results from the reaction between anchored, or cellular, antibody, and antigen. This observation was the foundation of the cellular theory of anaphylaxis. It was generally accepted, and the cellular theory was generally considered valid until the year 1909. In that year both Friedemann and Friedberger attacked its general validity, since which time practically the entire German school has either rejected the cellular theory entirely, or has accepted it as only part of the explanation of anaphylactic shock, maintaining that the reaction of antibody and antigen in the serum also constitutes an important factor. The observations upon which the new theory was based were the following: In the first place, it was found that in rabbits the simultaneous intravenous injection of antigen and of antibody could induce immediate anaphylactic shock and death (Friedemann). This same observation was then expanded so as to include guinea-pigs likewise (Biedl and Kraus, and others). The antigens so employed have comprised not only foreign serum, but also alien red blood cells, both in rabbits (Friedemann) and in guinea-pigs (Thiele and Embleton). Thus it appeared to be clearly demonstrated that the anaphylactic reaction might occur as a result of the combination of antibody and antigen in the blood, the cells playing absolutely no rôle in the reaction. But Friedemann and Friedberger went still further. They showed that if antigen and antibody were mixed in proper proportions outside the body and incubated for a short time, the intravenous injection of the mixture at once produced anaphylactic shock both in rabbits and in guinea-pigs. To the hypothetical poison so produced Friedberger gave the name "anaphylatoxin"; it had previously been described under the name of "apotoxin" by Richer. Friedberger and his school now maintained that the identical substance was produced *in vivo* when the antigen is injected into a sensitized animal, and that the reaction invariably takes place in the blood. Such is the foundation of the "humoral theory" of anaphylaxis.

From 1909 until the present time there has been this split upon the question concerning the site of the anaphylactic reaction. At first thought it might appear as though the discussion revolved upon a matter of no very great significance. The reverse, however, is the case. The interpretation of the rôle played by the immune bodies of the serum is wrapped up in the solution of exactly this problem. According to those who hold the cellular theory of anaphylaxis, the circulating antibodies protect the cells against shock. According to the humoral view, the circulating antibodies are the agents either of shock, or of protection, depending upon their amount in the blood. The whole problem of the mechanism of immunity is here involved and this explains the intense interest which recent research has given to the question. In his well-known review of anaphylaxis, carried up to November, 1912, Doerr summarized the evidence on each side impartially, but nowhere permitted himself to express even a guarded opinion as to the real solution. Since that date, however, he has made a very strong statement based upon later evidence, to which I shall subsequently refer. In a recent book on immunity, the situation is summarized as follows: "When antigen is injected into a sensitized animal, it is likely that it goes into reaction with both the circulating and sessile antibody. The latter is probably most important in the ordinary reaction of anaphylaxis, the former is probably of great importance in such cases as the sudden death which can be seen in highly immunized rabbits. . . . Both the cellular and the intravascular reactions probably occur in all cases." This evident uncertainty has, I believe, been terminated by the work of the last two years.

In the first place, a careful analysis of the recorded instances of anaphylaxis due to the simultaneous injection of antigen and antibody entirely fails to support the contention that the anaphylactic reaction is humoral. Every well-attested observation of that sort in the literature is based upon the injection of combinations of antigen and antibody previously mixed in the test tube. This is true both of rabbits and of guinea-pigs (Weil, Friedemann). Now there is ample ground for the belief that such mixtures may form toxic substances. Bordet, indeed, has shown that the incubation of normal guinea-pig serum with solutions of plain agar, or of starch, results in a product extremely toxic to guinea-pigs. But it is altogether unwarranted to draw the conclusion that the same processes occur when the two factors are separately injected into the blood stream of the living animal. As an actual fact, careful experimentation entirely fails to support this belief. In this connection, I may quote certain observations included in a previous paper of mine. "According to the terms of the humoral theory, it ought to be possible, by properly gauging the amounts, to introduce into a normal guinea-pig such a combination of antigen and of antibody (of foreign proteid and of serum from an animal immunized thereto), as at once to precipitate the toxic phenomena of anaphylaxis. An experiment was therefore planned in such a manner that a series of animals received a wide range of combinations of these two factors. . . . antigen and antibody being given simultaneously; in another series, the same combinations were employed, but the injections of antibody and of antigen were separated by a time interval. In the former case anaphylaxis failed to supervene; in the latter, it invariably occurred.

"These facts are illustrated in Tables I and II.

TABLE I

Showing that the Simultaneous Introduction of Antibody and Antigen is Ineffective

Guinea Pigs	Immune Serum 328	Horse Serum	Results
1.	0.1 c.c.	0.01 c.c.	No symptoms.
2.	0.1 "	0.1 "	" "
3.	0.1 "	1.0 "	Malaise.
4.	0.5 "	0.01 "	No symptoms.
5.	0.5 "	0.1 "	" "
6.	0.5 "	1.0 "	Malaise.
7.	1.0 "	0.01 "	No symptoms.
8.	1.0 "	0.1 "	" "
9.	1.0 "	1.0 "	" "

"Immune serum 328 was obtained from a rabbit, immunized by repeated intravenous injections against horse serum, and sensitized guinea-pigs in amounts of .1 cubic centimeter when introduced intraperitoneally.

TABLE II

Showing that the Same Amounts of Antibody and of Antigen used in Table I, Produce Anaphylactic Death when a Proper Interval Elapses Between the Two Injections

Guinea-pig	Immune Serum 328	Two Days Later Horse Serum	Results
1.	0.1 c.c.	0.01 c.c.	Death.
2.	0.1 "	1.0 "	"
3.	0.5 "	0.1 "	"
4.	0.5 "	1.0 "	"
5.	1.0 "	0.5 "	"
6.	1.0 "	1.0 "	"

"In Table I the guinea-pigs were injected with varying amounts of horse serum, and of serum derived from a guinea-pig highly immunized to horse serum. The immune serum was in these experiments injected into one jugular and the horse serum immediately thereafter into the opposite vein. In none of the animals were anaphylactic symptoms induced; in those which received 1 c.c. of horse serum there was slight evidence of discomfort. In Table II, the guinea-pigs received an injection of immune serum derived from the same animal that furnished the serum used in the previous set of experiments. After an interval of forty-eight hours they received an intravenous injection of horse serum. In every case death resulted immediately with convulsions. If the amounts of antigen and of antibody employed in the two sets of experiments be compared, it will be found that they exactly coincide. These two sets of experiments, taken together, demonstrate that some change must occur in the injected guinea-pig before it becomes sensitized; in other words, that in addition to the relative amounts of antibody and of antigen that enter into the reaction, there must be some additional factor which conditions the production of anaphylactic shock."

We must, therefore, accept the latent period as an essential feature of passive anaphylaxis. Its explanation, however, has given rise to further discussion. The theory was advanced by Biedl and Kraus that the latent period of passive sensitization was occupied by the "activation" of the *circulating* antibody, in such wise that it was made capable of reacting with antigen to produce shock. This, they maintained, was the explanation of the latent interval, and not the fact, as suggested by Otto, that the cells anchored the antibody. It was a very simple matter to test, and to disprove this theory. If a guinea-pig be passively sensitized with a large dose of immune serum, and be exsanguinated after 24 hours, enough antibody still remains in circulation to sensitize another guinea-pig. But the latent interval is not abolished, or even shortened. This observation, which I published some time ago, has since been amply confirmed in a paper by A. F. Coca, and quite effectively disposes of the explanation offered by Biedl and Kraus.

On the other hand although no qualitative changes can be shown to occur in the circulating antibodies, quantitatively they undergo a very striking change. Almost immediately there is a marked decrease in their amount, which progresses steadily for several days. This fact, which was brought out in my own experiments, has more recently been corroborated by very careful experiments of Fenyvessy and Freund. Eventually, after about four days, the antibodies may practically disappear entirely from the blood.

Now the question arises, *What has happened to the antibodies?* In spite of their decrease in the blood, in fact coincident with this diminution, the anaphylactic state of the animal develops and increases in intensity. For several days following the complete disappearance of antibodies from the blood, the passively sensitized animals continue to be hypersensitive. Inasmuch as the anaphylactic state unquestionably depends upon the presence of antibodies in the body, it is evident that the antibodies cannot have been lost. There is only one possible explanation of the facts. The antibodies have disappeared from the circulation, but they have been anchored by the cells. Thus the serological analysis of the latent period establishes the truth of the cellular theory. Doerr, in reviewing this evidence, has accepted the same interpretation, and speaks of it as an "emancipation from current beliefs."[1] Thus within a year of the publication of his reviews in Kolle and Wassermann, he abandoned his non-committal attitude and embraced the cellular theory.

There are certain other data to which reference should be made in this connection. Manwaring, Pearce and Eisenbrey, and Coca have made use of transfusion to test the theories previously outlined. They reasoned that if sensitized animals, which had been thoroughly exsanguinated and then transfused, still reacted to the antigen, this was evidence that the reaction was produced through the anchored antibody. Coca and Pearce found that this was actually the case in their experiments. Friedberger, however, has very properly objected that it is impossible completely to wash out the vessels, and that the remnant of fluid in the tissue spaces might well mediate the reaction. Doerr has declined, and I think rightly, to accept this type of evidence as final.

A very important contribution to the subject was made by means of the use of isolated tissue. Schultz, in Washington, first showed that strips of intestine taken from a sensitized animal respond with a vigorous contraction to the application of the antigen. Dale improved upon this technique by substituting the isolated uterus. Moreover, Dale showed that a uterus, of which the vessels had been thoroughly perfused with Locke's fluid after death, still responded to the antigen. The objection was raised by Friedberger that perfusion did not wash out the tissue spaces, and that the antibody here present might well be responsible for Dale's results. Even this last possibility, however, has been excluded. I have shown that if a guinea-pig is given an intravenous injection of large amounts of antibody, and is killed within an hour, the uterus fails to respond. In such an experiment there is no doubt that all of the tissues are thoroughly bathed by the injected antibody, yet the anaphylactic condition does not supervene.

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[1] *Ztschrift. f. Immunitätsforsch.*, 1909, xix, p. 290.

The whole subject may be summed up, therefore, by the statement that *all the evidence proves conclusively that anaphylactic shock is induced by reaction between anchored antibody and antigen, and that circulating antibody plays absolutely no rôle in its production.*

The previous discussion has been based upon the use of antigen in the form of soluble proteins, such as blood serum, milk, or egg albumen. It still remains to consider the case of formed antigens such as red blood cells. Without going into the evidence in detail, it will suffice to say that with this condition also, the reaction is always intracellular. The divergent results hitherto recorded are based upon errors of technique. A detailed discussion on this subject may be found in a forthcoming paper on red cell anaphylaxis by Dr. Denzer and myself.

The question now arises as to why the intracellular reaction should be provocative of such intense symptoms, whereas the intravascular reaction is practically harmless to the organism. The answer to this question is to be found in an analysis of the latent period of passive sensitization. It has generally been held that the latent period is concluded when the cells have absorbed sufficient antibody to complete their sensitization. This is the quantitative theory of the latent period. Recently I have analyzed this view by means of the following experiment: Guinea-pigs were given a sensitizing dose of the serum of a rabbit immunized against horse serum. Before the lapse of the latent period, these guinea-pigs were killed, and both horns of the uterus suspended in the Dale apparatus. One horn was immediately tested with horse serum, and on failing to respond was carefully washed. Both horns were then kept at body temperature in oxygenated Locke's fluid for several hours. At the end of this time, both responded to the addition of horse serum. But the response of the previously tested horn was distinctly less prompt than that of its opposite fellow. These results permit of certain definite conclusions. During the first part of the latent period the body cells absorb antibody from the serum. This antibody can unite with antigen, as is shown by the fact that the second reaction obtained from the previously tested horn is characteristically that of a partially desensitized uterus. Thus the union of cellular antibody with antigen is possible during the first phase of the latent period, but does not induce a typical cellular discharge. The second phase of the latent period is occupied by the activation of the cellular antibody, in such wise that its union with antigen produces a typical anaphylactic response. This interpretation is in complete harmony with the observation of Fenyvessy and Freund, who showed that sufficient antibody to sensitize the animals disappears from the blood during the early phase of the latent period; yet sensitization is not complete until a still longer interval. Cellular activation adds still another feature to anchored antibody, in that it greatly increases its avidity for antigen, over that possessed by circulating antibody. I have shown this in a previous paper concerning avidity in passive sensitization, and the same fact holds of active anaphylaxis.

Finally, a word as to the so-called "toxic manifestations" of anaphylaxis. I believe that as yet no toxic effect has been demonstrated. The anaphylactic reaction represents simply a cellular discharge of many of the tissues, including the nervous system and the bronchial musculature. The death induced by this discharge is accidental. If the animal recovers, he may be practically at once in normal condition. In the same way, with the Dale method we can induce a series of uterine contractions with a series of antigens, the muscle being throughout in unaltered condition. Thus the reaction is of the nature of a physiological response to a stimulus. There is no known toxin which acts in this manner, either as regards the promptness of its action, or of the recovery of the animal.

Discussion:

Dr. Zinsser: The subject of Dr. Weil's paper is too extensive to be thoroughly discussed in the time available. There are, however, a number of important points in which one might take exception to Dr. Weil's opinions. I think that the idea that anaphylaxis is primarily a cellular reaction has been foremost in the minds of many investigators since the work of Dale and Schultz and since the experiments of Pierce and others done by the transfusion method. Most of us I think believe that the meeting of antigen and antibody in the circulation cannot alone cause the violent reactions observed in the typical anaphylactic experiment. However, symptoms have been obtained by the simultaneous injection of antigen and antibody by Friedmann, Biedl and Kraus and a great many others. It is true that symptoms produced in this way have usually been less violent and have been delayed. Dr. Weil objects to calling this anaphylaxis, because the typical anaphylactic death was not produced. I do not think that this is justified, since it does not seem logical to define anaphylaxis only by its most severe and acute manifestations.

I agree with Dr. Weil that it is extremely difficult to obtain responses in guinea pigs or rabbits when the interval between the injection of the two is not observed. Personally I have had distinct symptoms when injecting antigen and antibody together, although I have not been able to repeat exactly the work of Friedmann, nor that of Biedl and Kraus.

I believe, indeed, with Dr. Weil that acute anaphylaxis is chiefly a cellular reaction. I believe at the same time, however, that there is injury done the body when antigen and antibody meet in the circulation in definite proportions. This matter of definite proportions, however, is very important. The two do not unite readily in the circulation. It is well known to most of you that in animals treated with any antigen, the antigen and antibody may be present in the circulating blood side by side without having united. Why should this be possible? Many explanations have been offered for this by Eisenberg, von Dungern and others. We do not believe that their explanations are as likely as one advanced by us a few years ago, namely that the lack of union is due to colloidal inhibition possibly to the presence of the protective colloid.

Together with Young some years ago I was able to produce with gelatine, gum arabic and arsenic trisulphide an analogy to the conditions in serum, which persuaded me that conditions in serum might be very similar to those in the colloids mentioned. Gelatine precipitates the arsenic. A little gum arabic will prevent such precipitation. When two such balanced solutions are spilled one in the other, precipitation takes place since the relative proportions necessary for protection are disturbed. This is true also of two sera in which antigen and antibody are both present but have not united.

There is no time to go into these relations more thoroughly. I wish simply to say that I think the symptoms produced by the simultaneous injection of antigen and antibody into an animal are delayed and slight because of some such inhibition to their union. When quantities are such that slow union can take place, symptoms result and this I think accounts for the results obtained in this way by many experiments of many workers, which I do not think should be entirely ignored and which certainly should prevent one from completely eliminating intravascular reactions in the way that Dr. Weil does.

Dr. Weil: If anaphylactic symptoms result from the union of antigen and antibody *in vivo,* it ought to be possible to produce the same result by the simultaneous injection of these substances into the circulation. Dr. Zinsser states that he himself has never been able to produce death in this way. As an actual fact none of the writers who have worked with serum as antigen have ever succeeded in reproducing the characteristic picture of acute anaphylactic shock by that method. The fact that the previous mixing of the antigen and antibody in the test-tube does produce this result, is sufficient evidence that the preliminary procedure must have induced some toxic alteration, even though it is not visible as a precipitin.

I have stated that there is no satisfactory evidence that the simultaneous injection of antibody and of antigen, either as serum or as red blood cells, can produce anaphylactic symptoms. Dr. Zinsser has quoted in support of the opposite contention the observations of Briot, of Scott, of Biedl and Kraus, of Friedemann, and finally of Gurd. Most of these citations are frequently found in the literature in this connection, and it is therefore worth while to examine them in detail.

Briot states definitely that his injections of rabbit versus horse serum, and of horse serum, were separated by an interval of 24 hours.

Scott states that simultaneous injections give rise to symptoms which are distinctly milder and more transient than those obtained by spaced injections. His statement covers about five lines, and is supported by no data.

Biedl and Kraus simply state that simultaneous injections are " wirksam," but give not a single experiment.

Friedemann states distinctly that his injections of serum and of antiserum were *mixed.* Concerning his experiments with red cells, he himself admits that red cells hemolyzed with distilled water also produce the same toxic symptoms when injected alone.

Gurd worked with rabbit versus sheep serum, which is well known to be highly toxic intravenously to guinea-pigs. He made not a single intravenous control with this serum. Even so, he succeeded in producing death in only five of his animals, of which none died in less than one hour, and two died next morning.

Thiele and Embleton worked with rabbit versus sheep red cells, one of the most toxic antisera known.

I believe, therefore, that I am justified in concluding that there is not in existence a single set of experiments which give satisfactory evidence that anaphylactic symptoms can be produced by the simultaneous injection of antibody and of antigen. In every instance, accessory factors, such as the primary toxicity either of antiserum or of antigen, have played a complicating rôle, not excluded by the experimental method. But even so, the results have been very irregular, so as to partake, as Doerr says, of the nature of accidents.

Dr. Zinsser admits that acute anaphylaxis has not been produced by simultaneous injections, but asks us to accept the type of results hitherto reported as evidences of a mild anaphylaxis. This is, of course, somewhat illogical. If it has been possible to induce mild anaphylaxis, it should be a very simple matter, by increasing the amounts of the interacting substances, to multiply the toxic product. The failure to accomplish this, again supports the belief that the so-called mild anaphylaxis of simultaneous injections is in reality something altogether different.

When it has once been definitely proven, that the simultaneous injection of antigen and antibody, in themselves not highly toxic, can regularly be made to induce typical anaphylactic symptoms, the time will have arrived to revise the conclusion which I have supported.

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DR. HANS ZINSSER, *President*

THE HISTOPATHOLOGY OF CALCIFICATION OF THE SPINATUS TENDONS, AS ASSOCIATED WITH SUBACROMIAL BURSITIS

ELI MOSCHCOWITZ, M.D.

The lesion is a common one. It is a frequent cause of shoulder disability and, as such, it has long been and still often is mistaken for brachial neuritis, rheumatism, etc.

In this affection the shoulder is stiff and painful, the symptoms and the lesion itself developing soon, as a rule, after trauma. Neither the pain nor the stiffness is characteristic in extent or incidence. The pain may be continuous or intermittent, spontaneous or on motion referred to the shoulder itself, or, more often, to the outer or anterior aspect of the arm. It sometimes radiates into the forearm or the neck. The stiffness may be manifested by awkwardness or by pain on motion. There is usually limitation of abduction, sometimes moderate, sometimes extreme. There is usually also limitation of rotation, internal, external or both. There is often tenderness in the upper deltoid region.

X-ray photographs of the cases studied showed well-defined areas of calcification within the spinatus tendons. These masses vary in size from a pea to a flattened nodule measuring 3–4 cm. in length. The masses may be single or multiple. The shadows are situated on the outer side of the tuberosity or head of the humerus, extending to beneath the acromion process.

PATHOLOGICAL EXAMINATION

The material studied consisted of tissue removed from three cases by Dr. Brickner.

CASE 1. A portion of the supraspinatus tendon removed sixteen days after injury. Section shows a crescentic mass of tissue. Covering the border is a fairly well organized granulation tissue. Within this zone and filling the entire cavity of the crescent is richly cellular early granulation tissue consisting of many round cells and plasma cells. There are a large number of newly formed cells and the remains of a recent hemorrhage. Filling the cavity, of which this section is an arc, is a mass of amorphous necrotic tissue in which are discrete deposits of lime.

Summary.—This section reveals substantially two stages of granulation tissue; an outer, the older, and an inner more recent variety. These inclose a necrotic focus which has partly undergone calcification.

CASE 2. Removed four months after injury. Due to imperfect orientation two separate masses are seen in the section. The first and larger consists of tendon fibers that have undergone hyaline degeneration in parts and in other parts calcification. From one aspect of the tendon there has sprung a large mass of granulation tissue, which is richly cellular, almost sarcomatous in appearance, consisting of large spindle shaped connective tissue cells with little fibrillar intercellular substance. In this tissue there are numerous newly formed young blood vessels. There is abundant lime within this granulation tissue in the form of sharply circumscribed discrete masses; some of the masses are in immediate relation to giant cells.

The second and smaller mass consists of hyaline tendinous tissue which has undergone almost complete calcification.

Summary.—A tendon that has undergone extensive hyaline degeneration and calcification, with manifest necrosis and secondary calcification of the tendon. In addition there is recent granulation tissue, in which are imbedded numerous calcified granules.

CASE 3. Removed one and a half years after injury. The tendon shows essentially a mass of tendon tissue enclosing a cavity containing necrotic detritus. Between the tendon bundles there is abundant firmly organized granulation tissue. While many of the tendon bundles have undergone hyaline degeneration the necrotic tissue either is loosely within the cavity or is directly continuous with the surrounding tendinous area. No calcification is discoverable, although Dr. Brickner assured the writer that abundant gritty masses were found at the operation; the lime must have been shaken out of the specimen during transport.

Summary.—A tendon that has undergone partial hyaline degeneration and is infiltrated with abundant connective tissue. Necrosis of tendon.

DISCUSSION

These specimens exemplify three progressive stages of a lesion that may be termed a "tendinitis."

The first common factor is the formation of a new granulation that corresponds, according to morphological criteria, to the duration of the illness.

A second common factor is the presence of necrotic foci, which in two of our three cases were demonstrably calcified, and in the third probably so. The explanation of the necrosis is obvious. The blood supply of a tendon is notoriously meager so that only a slight tear or injury is necessary to completely cut off the blood supply.

The pathogenesis of calcification within tendons is governed by the same conditions that apply to abnormal calcifications in other parts of the body. It has been definitely established that calcification occurs only in dead or inert tissues. It has thus a peculiar predilection for tissues that are necrosed or that have undergone hyaline degeneration.

The early calcification that has been noted in these cases (Dr. Brickner has seen cases in which X-ray shadows were demonstrable three days after injury) is remarkable, and can only be explained by the observation of certain experiments upon pathological calcification in other tissue of the body. For instance, Litten and Lick found lime in necrotic kidneys one day after ligation of the renal artery. Especially significant are the observations of Schujeninoff, who found calcification of muscle fibers 36 hours after suture of the divided muscle. Numerous experimental observations upon the healing and regeneration of tendons have been made, but these fail to throw any light upon the problem of calcification of tendons. This may be due to the fact that nearly all the observations were made upon the Tendo Achilles which may prove to be structurally different from the spinatus tendons. At all events, I am not aware of any disease of other tendons analogous clinically to the condition under discussion. In other words, the associated symptom-complex, namely, trauma, necrosis and early calcification of a tendon, attended by protracted disability, is apparently nowhere observed except in the spinatus tendons.

The only conditions to which this malady may be likened are the comparatively uncommon traumatic "ossifications" discoverable by X-ray in various tendons, especially around joints. To these may be added the "calcaneus sporn" arising from the posterior surface of the os calcis and extending into the Tendo Achilles; "Rider's bone" or "ossification" of the adductors of the thigh; and "Drill bone," or "ossification" at the insertion of the deltoid. Myositis ossificans is in all probability an allied condition. The possible objection that these shadows are bone and not lime does not entirely disqualify the parallelism. In the first place, pathological evidence that these shadows are bone and not lime is still wanting. In the second place, it is now generally conceded that pathological ossification cannot occur without premilinary calcification, so that these shadows in their early stages must have represented deposits of lime. These lesions, however, cannot be compared either in the matter of frequency or in the early development of the lesion.

The lesion described in the spinatus tendons has, however, certain precise pathological analogies. We refer to the rare multiple nodes described in tendons by Krause and Trappe, Neuwirth and L'Hermitte, which are embolic in origin and are probably due to calcification of an infected focus.

The gouty deposits in tendons are also identical pathologically, the only difference being that instead of lime the deposit consists of urates.

Discussion:

Dr. Brickner: Dr. Moschcowitz's presentation is especially interesting because it is the first systematic histologic study of calcareous deposits in the spinatus tendons and is based on the largest series of cases that have been studied surgically. I think we may say that we now have a clear notion of this variety of "shoulder disability" in its histologic and surgical aspects, but there remain many problems to be solved. Among these is the peculiar tendency of the supraspinatus, sometimes the infraspinatus tendon, to the early deposit, sometimes large and multiple deposits, of lime salts, after comparatively mild violence. I have seen twenty cases in the last couple of years.

I am not sure that Dr. Moschcowitz is justified in calling calcifying spinatus tendonitis the chief cause of shoulder disability, but I believe subacromial bursitis, which often has this tendon lesion associated with it, *is* the most common cause.

I use the term "shoulder disability" in preference to "stiff and painful shoulder" because either the pain or the stiffness may greatly preponderate or exist alone.

Codman first gave a very comprehensive description of subacromial bursitis, anatomically and clinically, and in two articles correctly located one of the varieties of deposit—the semi-fluid, extratendinous—as beneath the bursa, but he spoke of it as of a cyst, which is wrong, for there is no capsule or limiting membrane. Wrede, in the report of a single case, also located the deposit correctly, viz., in the supraspinatus tendon. Aside from these two, all writers on subacromial bursitis and tendon deposit have been in error. Indeed, the literature of this subject strikingly indicates the not uncommon tendency in medicine to jump at wrong conclusions, and the frequency with which fallacies are transmitted from one author to another.

Painter, who was the first to observe the calcareous deposit, reported that he evacuated it from within the bursa, although Codman, who was present at the operation, observed that it came from beneath the bursa. In two other cases operated upon by Painter he found no deposit (because he did not look beneath the bursa), and he assumed that the X-ray shadow was produced by thickening of the bursal walls. Since then all surgeons and radiographists, excepting Codman and Wrede, have regarded these shadows as due to thickenings of, effusions in, or calcifications of the bursa. Holzknecht, for example, calls the calcareous mass a "bursalith." Cumston recorded one of these cases as a suppurative bursitis, although there was none of the familiar symptoms or signs of suppuration, and in spite of the fact that the "thick, yellowish pus" evacuated "from the bursa" was not examined. When, as is often the case, the bursal walls are adherent, an incision into the sac, unless made with this consideration in mind, is apt to go through the floor of the bursa, evacuating from beneath the often cheesy deposit, which thus appears to come from within the bursa. Baer, who also operated upon cases of subacromial bursitis not only repeats the error that "replacement of the bursa by scar tissue causes a shadow in the radiogram," but also assumed that "if the process is a tuberculous one the cavity may be filled with a dense, cheesy material." I have seen but a single, doubtful case of tuberculous subacromial bursitis. I have had one of these "cheesy" calcareous deposits studied bacteriologically; it was not only germ-free (smears and cultures), but also produced no reaction in guinea-pigs.

Another fallacy has been the assumption of bacterial or toxic irritation as an etiology of subacromial bursitis. Such an assumption has never been substantiated. If the condition were bacterial or toxic we ought to see it also in the olecranon, trochanteric and other bursæ. Excluding the rare tuberculous and the occasionally suppurative (septic) variety, subacromial bursitis (the bursitis of shoulder disability) is traumatic in origin, due, I believe, to bruising the bursa and the tendon between the head of the humerus and the acromion process. The trauma may be either an external or an internal violence. As instance of an external violence, a fall upon the outstretched arm, which jams the humerus against the acromion. A physician whom I operated upon this week, thrown by an automobile *upon* his shoulder (he says) seventeen days previously, had dense adhesions in the bursa and two discrete, large, dry, powdery calcareous deposits in his supraspinatus tendon. Very often there is no history of injury. In these cases the lesion results from mild internal violence, viz., a sudden or unduly vigorous abduction of the arm, as in the lurch of the body while hanging to a street-car strap, exercising on flying rings, leaning on the outstretched arm as in scrubbing the floor, etc. In all these forms of injury the bursa and tendon (supraspinatus, infraspinatus, or both) are compressed between the two bony surfaces. There results, as may be seen at operation, a hemorrhagic infiltration of these structures and, often, a small tear of the supraspinatus near its insertion, sometimes through its entire thickness. There is no doubt that a tear of this tendon can be produced by sudden abduction, in the adult. Codman reported three, and operated upon two, cases of complete rupture of this tendon. In one, a woman, it resulted from throwing a heavy blanket over a clothesline; in the other, a man, from tightening a saddle-girth. In both cases something was felt to snap, and the arm fell limp.

There are two forms of the calcareous deposit, in my experience,—one chiefly extratendinous, and associated with a tear in the tendon; the other, single or multiple, entirely within the tendon and with no surface indication of tendon injury. Either form, independent of the acuteness or chronicity of the case, may be semi-fluid or dry and gritty. While Dr. Moschcowitz is no doubt correct, as a pathologist, in calling this a "calcification of the tendon," surgically it is to be regarded only as a calcareous deposit. There is no extension, grossly, of the calcification beyond the torn fibers immediately surrounding the deposit.

We have both referred to the early appearance of the lime. I have operated upon cases sixteen and seventeen days after the injury, and I have seen the shadow of the deposit ten and five days and even, if the history was reliable, one day after injury.

The operative plan I follow consists in splitting the deltoid muscle, carefully incising the bursal roof, dividing all bursal adhesions, incising and dissecting up the floor of the bursa, removing the deposit from the surface or from within the tendon (usually the supraspinatus), trimming the edges of the tendon tear or incision, suturing the tendon, and then suturing, successively, the floor of the bursa, its roof, the muscle, and the skin, without drainage. I smear a very thin layer of vaseline in the bursa. The arm is abducted in plaster for about eight days.

GRAM-NEGATIVE MICROCOCCUS CAUSING FATAL ENDOCARDITIS

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The presence in the blood stream of the gonococcus and meningococcus as a general bacteriæmia with septic manifestations has been observed repeatedly. In the case to be reported here, there was presented the familiar clinical picture of infective endocarditis with the recovery from the blood during life, and from the organs at autopsy, of a Gram-negative micrococcus which appears to be closely related to the gonococcus, but differs from it in important particulars, and represents probably a new species.

The patient, a Swiss cabinet-maker of 45 years, entered the Presbyterian Hospital November 6, 1914, suffering with dyspnœa and general weakness and repeated vomiting. The onset of symptoms had occurred three months previous with chill, followed by progressive weakness and night sweats. The patient presented the signs of cardiac enlargement with a loud systolic murmur, and pulse of auricular fibrillation. The liver and spleen were enlarged. No petechiæ were found. Diagnosis of malignant endocarditis was made.

Blood Examination, Nov. 7.—Leucocytes, 8100. Polys., 69 per cent.; Monos., 28 per cent.; Eosin., 3 per cent. Blood culture, positive.

Disease ran an irregular febrile course, temperature reaching 103°–4°. There were recurrent attacks of tenderness over the spleen and associated with this on November 17 there was swelling and redness over the 5th left metatarso-tarsal articulation, and on December 2 swelling and redness appeared over right 1st metacarpo-carpal joint, with tenderness of pulp space of adjacent forefinger.

November 17.—Gonococcus complement fixation serum, anticomplementary.

November 27.—Leucocytes 12,200. Polys., 79 per cent.; Lymphos., 29 per cent.

December 7.—Leucocytes, 11,400. Polys., 81 per cent.; Lymphos., 18 per cent.; Eosino., 1 per cent.

Blood cultures were positive on November 7, November 11, November 13, November 27, November 30 and December 16. Last culture plates showed 6 colonies per c.c.

December 25.—The patient developed rather suddenly a left hemiplegia and died after about 36 hrs. with signs of cerebral hemorrhage.

At *autopsy,* 8 hrs. post mortem, was found an early bronchopneumonia of both lungs. The heart was large, weighing 520 grams, and showed hypertrophy and dilatation. The valves were normal, except for the mitral which showed on the margin of its posterior cusp a rounded and thickened tag of tissue, just large enough to contain a circular ulcer 6 mm. in diameter. This ulcer completely perforated the valve; its margins were firm and raised, its cavity filled with fibrin; in one spot it showed a minute vegetation. This small patch was the only evidence of vegetative endocarditis throughout the heart.

In the wall of the jejunum was found a small thrombotic nodule, 8 mm. in diameter. The liver and kidneys showed a state of chronic passive congestion. The pelvis of the right kidney contained pus. In several of the kidney glomeruli were seen in sections necrosis of a portion of a glomerular tuft, and later stages, with fibrosis and adhesions to the capsule, of the lesion considered characteristic of *Streptococcus viridans* endocarditis.

On a branch of the right middle cerebral artery was found an embolic aneurysm 7 mm. in diameter. Rupture of this had caused an extensive hemorrhage into the brain tissue, and had been the immediate cause of death.

Cultures at Autopsy.—Heart's blood: sterile. Kidney: sterile. Pyelitis: sterile. Lung: streptococcus and pneumococcus mucosus. Hemorrhage in brain: same organisms as found in lung. Spleen and the embolus in the jejunum: pure culture of Gram-negative micrococcus. Section through the ulcer on the mitral valve shows clumps of small cocci lying in the zone of fibrin just beyond the necrotic valve margin, and reaching the surface of the ulcerated area in one spot.

DESCRIPTION OF ORGANISM

The microorganism isolated appears as a very small coccus, in size equal to the smallest forms seen in the ordinary *Staphylococcus aureus.* There is hardly any visible variation in size between individuals in a given culture and in growths on the various media. Diplococcus forms are frequent and resemble in form the biscuit-shape of the gonococcus. Small clumps of three to eight are most numerous. Curious bacillary forms occur, the shorter of which can be resolved into a chain of four or five cocci, the larger appear homogeneous, and reach twice that length.

Stained by Gram's method with the meningococcus and hay bacillus on the same slide as controls, this coccus is invariably decolorized. Methylene blue and safranin alone stain it poorly. Fuchsin gives a more intense stain.

The first isolation from the blood was obtained on 1 per cent. dextrose-agar plates, poured at the bedside, but growth did not occur in inoculations into dextrose-meat-infusion-bouillon. Attempts at isolation from the blood were made by other investigators, using media of the same formula, but were never successful. On blood plates the colonies appeared after 48 hours incubation at 37° C. Typical colonies were 0.5 mm. in diameter, dark green in color and surrounded by a diffuse zone of light olive green. On surface cultures the *characteristic delicate growth* is visible after 12–24 hours, and reaches its maximum at 48 hours. Colonies are always discrete, and macroscopically, are of two types: The smaller and more numerous are minute and almost invisible to the naked eye except when crowded together, where they give the appearance of a confluent growth. The larger colonies attain a diameter .25 mm., and appear as glistening transparent droplets. Microscopically, the colonies are alike, they are roughly spherical, dry, and appear made up of minute refractile granules. When a colony is touched with the needle it is easily dislodged, and moves about on the surface of the medium without breaking up.

Simple dextrose-agar was used successfully for the isolation from the intestinal embolus, and transplants in the second generation from the blood plates grew well in this medium. Growth did not occur at first on Loeffler's blood serum, but does so after artificial cultivation. Ascitic dextrose-agar is the most favorable medium, and after continued cultivation upon it the separate colonies are opaque, white, with wrinkled surface and outline, and tend to umbilication. They resemble minute bread crumbs. Under cultivation there is no increase in size of colonies. On the simple solid media no change has occurred, and growth appears as on first isolation.

In fluid media and in the water of condensation of solid media, growth is peculiar. After 8–12 hours discrete granular masses up to 0.5 mm. in diameter are noticed in the bottom of the flask, clinging to the sides of the glass. The supernatant fluid is perfectly clear. After 36 hours a general cloudiness begins and increases for 2–3 days. Old broth cultures show a delicate milk-white veil adhering tenaciously to the bottom and sides of the glass and a few delicate filaments springing from the veil float in the fluid.

Growth is as vigorous anaerobically as aerobically, and neither macroscopically nor microscopically do the two cultures differ.

Microscopically this organism appears like a minute gonococcus or meningococcus, but large involuted forms, metachromatic staining, and rapid autolysis, do not occur. The isolation upon serum-free media, and the vigorous growth throughout bouillon tubes, are important points of differentiation from the gonococcus. The firm dry colonies and spontaneous sedimentation in broth cultures make the organism resemble the *Micrococcus pharyngis siccus*, but the extreme delicacy of growth of this unclassified coccus is unlike that of any of the Gram-negative micrococci with which I am familiar.

Dextrose is the only sugar fermented. In litmus milk there is a slight change in color, but levulose and galactose used alone are not acidified. Thus the fermentation reactions suggest the gonococcus.

An immune serum was produced by intravenous injections of killed cocci into rabbits. This agglutinated the coccus in a dilution of 1 : 32 and very slightly in 1 : 64. This serum was tested against a strain of meningococcus with entirely negative results. A strain of gonococcus was agglutinated in 1 : 32 dilution. Portions of serum were then absorbed with the homologous coccus and the gonococcus. Serum absorbed with its specific coccus no longer agglutinated the gonococcus while after absorption with the gonococcus, it still agglutinated the specific coccus in dilution of 1 : 16.

The coccus was not agglutinated by the patient's serum, nor by a commercial antigonococcus serum, which however gave negative results with the gonococcus strain tested above, nor with a meningococcus immune serum.

This coccus is highly pathogenic for mice. One fifth of the 48-hr. growth on a blood agar slant killed a white mouse in 4 hours. The coccus was regularly recovered from the heart's blood of experimentally injected mice.

Guinea-pigs succumb in 12 hours to large doses intraperitoneally; chronic infections have not been set up, using smaller amounts of material for inoculation.

Similar results have been obtained with rabbits; small doses do not set up a chronic infection; large doses intravenously result in death in 60 to 72 hours. The organism has not been regained from the blood during life of experimental rabbits, nor from the heart's blood at autopsy, and it seems probable that death in guinea pigs and rabbits is due to the liberation of a toxin from the bodies of the cocci.

Attempts have been made to obtain a soluble toxin, by passing bouillon cultures, grown from periods varying from 48 hours to 10 days, through a Berkefeld filter. The filtrates have been uniformly non-toxic for white mice and guinea-pigs.

CONCLUSIONS

Morphologically, this organism seems related to the Gonococcus-Meningococcus group. Culturally, it does not conform with any organism with which we are familiar, although here too there are suggestive points of resemblance to the well-known Gram-negative diplococci. Accurate differentiation on the basis of serum reactions can not be made until an immune serum has been developed with an agglutination titer higher than 1 : 64, the maximum so far obtained. Preparation of such a serum has been rendered difficult by the toxicity of the coccus for rabbits.

CASE OF SUB-LEUKÆMIC LYMPHADENOSIS

MORTIMER WARREN, M.D.

(Pathological Laboratory, Roosevelt Hospital)

The title given to this case report is used more in a descriptive than in a diagnostic sense in an attempt to define the pathological conditions found.

In order to arrive at any kind of a diagnosis it is, of course, essential to know as much as possible of the clinical history and course of the patient, especially since the objective evidence is limited to the histological appearance of one lymph node and to the blood findings. I therefore will give briefly the history and status of the patient.

Clinical History.—A white male of twenty-six years, born and brought up in the British West Indies,—a railroad clerk by trade,—entered The Roosevelt Hospital on June 5, 1914, service of Dr. Evan M. Evans, History No. D–6192. His chief complaint was weakness, gradually increasing for the past fifteen months; at times pallor and yellow color had been noted by his friends. For a week before admission he had some evening fever and slight sweats with chilly sensations. Past history is unimportant aside from that of malaria when in the British West Indies, with frequent dosage of quinine; he had, however, no chills.

Examination.—A young man of 116 pounds, with evident pallor and slight icterus. Moderate enlargement of axillary, inguinal and epitrochlear lymph nodes. Spleen edge is palpable 2¾ inches below costal margin. Liver edge not felt. Heart and lungs negative. No sore throat. There was a petechial spot in the soft palate which subsequently disappeared.

During his stay in the hospital from June 5 to July 3, 1914, examinations of urine were negative, aside from the presence of bile in one instance, and of urobilin in another. The gastric contents showed lack of free hydrochloric acid and the presence of occult blood. Fæces were negative for ova and parasites. Temperature was irregular ranging from 99° to 102°, but towards the end of his stay lessened in height. A blood culture was negative. Wassermann was negative.

On June 10, 1914, a pectoral lymph node was excised, section of which (B–7028) I wish to show and present for your opinion and diagnosis.

The *blood findings* were interesting, and were, I believe, directly related to the changes in the lymphatic apparatus as witnessed by the histological structure of the excised lymph node. Hæmoglobin, 54; leucocytes, 18,000; erythrocytes, 4,400,000. Polynucleus, 42.6 per cent. Lymphocytes, 12 ordinary lymphocytes; 19 stimulation forms; 18.6 pale cells. Transitional, 1. Eosinophiles 5.3. Mast cell, 1.3.

DISCUSSION

The leucocyte count was sub-leukæmic, being 10,000 to 20,000 with some forty-odd per cent. of mononuclears belonging to the lymphocytic series, with a constant increased per cent. of eosinophiles.

Anæmia was present at all times, more marked in the individual red blood cells than in the total count: of chlorotic type, with considerable pallor and variation in size, shape and staining reaction of the cells. Occasional normoblasts were seen. Platelets in smears were evidently increased.

At first we tried to make the leucocyte formula fit into the myeloid series but the individual mononuclears, though deeply basic in staining reaction of the protoplasm, were scanty in nucleoli and were non-granular. They gave no oxydase reaction and the blood was practically free from myelocytes or any cells which could be considered transitional forms in the neutrophilic group, though transitionals of the usual variety were present in fair number. The lymphocytes could be divided into three groups: (1) The ordinary small cell with its little protoplasm. (2) Large pale cells with azure granules,—likewise normal. (3) Abnormal deep, basic cells of the type of "stimulation" forms. Smears of the gland showed all the types of cells seen in the blood beside larger endothelioid cells.

Examination of sections led to a variety of diagnoses. I was inclined to some form of metaplasia at first,—later to the diagnosis of "Hodgkin's," a provisional diagnosis of which Dr. Mann had already made. This diagnosis was strengthened by the result of a growth from a portion of the node on egg media, which proved to be *Bacillus Hodgkinii*. Further study has, however, convinced both Dr. Mann and myself, as well as Dr. Cunningham, who has recently reviewed all our cases of Hodgkin's disease—some twenty in number—that this is not a proper diagnosis. Here I might say that Dr. Butterfield examined the smears of blood and sections of the gland and did not consider that it conformed to Dorothy Reed's description of Hodgkin's disease, but was unwilling to completely correlate the blood and tissue findings. A section of the gland, a smear of the blood and a plant of the culture were sent to Dr. Bunting in Wisconsin, who reported both the gland and organism typical of Hodgkin's disease. He regarded the blood differential as rather higher in lymphocytes than usual, except in early cases of the disease.

The gland itself shows a varying fibrosis which is marked only in portions and is somewhat whirling in character. The follicles as such are not evident; there are, however, focal collections of large mononuclears showing active mitotic division,—areas which may represent the germinal centers and the cells of which may be germinal center cells. Similar cells are also scattered throughout the gland in the interstitial tissue, and are likewise seen in the capillaries and in the lymph spaces. These cells appear to me to be the source of the abnormal cells, the lymphocytic stimulation cells of the blood. There are larger cells with well marked nucleoli occurring singly in the interstitial tissue; and in areas there are pale staining, endothelioid phagocytic cells, but there are no multinucleated giant cells, or giant cells with lobed nuclei. The preponderating cell is a lymphocyte of the small type. Occasional mast cells and scattered eosinophiles are present. I think I can see all sorts of transition stages between the large, actively dividing cell and the small lymphocyte, in certain examples forming a characteristic plasma cell.

I believe this is a case of leukæmia of atypical form: It is evidently not acute—patient was heard from about two months ago and was then in fair condition—and it is not of the chronic type, unless in an early stage. A count made one week after leaving the hospital showed 55,000 leucocytes.

One can readily believe the anæmia to be secondary, possibly in part hæmolytic in character, thus accounting for the icterus and the urobilinuria, though there were no definite signs of active regeneration. It is, of course, impossible to draw any conclusions as to the state of the bone marrow.

Discussion:

Dr. Zinsser: I would like to ask Dr. Warren's views on the possible relation of this case to cases in which *B. Hodgkini* is found.

Dr. Warren: We found this organism in a mediastinal sarcoma and in two or three tubercular lymph nodes. This one grew in pure culture in egg media.

Dr. Coulter: I have cultivated a similar organism in Hodgkin's disease from the cervical lymph nodes, and in another case similar to this one of Dr. Warren's, from excised lymph nodes.

Dr. Taylor: Doctor Pease has been making cultures at our hospital from various cases of glandular enlargement and has isolated by means of shake-cultures of glucose-ascitic-agar diphtheroid bacilli. These have been found in a number of cases of typical Hodgkin's and also in what was histologically lympho-sarcoma and a case which at autopsy was apparently tuberculosis. The possibility of a combination with Hodgkin's disease in this case could scarcely be eliminated, but it seemed to us that the whole process might be explained on a tubercular basis.

Dr. Warren: We have had frequent contaminations of blood cultures of a diphtheroid organism. Whether it is related to *B. Hodgkinii* I don't know.

Dr. Garside: In erysipelas I have taken the skin and rubbed it on the plate and got a pure culture of streptococcus. Dr. Bunting attempted to reproduce the disease, did he succeed?

Dr. Warren: He produced lymphatic enlargement and necrosis, but I don't think it is the same as in human beings.

Dr. Coulter: Did the case run a febrile course?

Dr. Warren: 99 to 102.

ADENO-CARCINOMA OF UTERUS WITH METASTASES IN BOTH TUBES

L. W. STRONG

(*Woman's Hospital*)

I have an interesting case of adeno-carcinoma of the body of the uterus with metastases in both tubes. So far as I am aware there is no case in the literature of a double metastasis in the tubes. There are, of course, cases of cervical carcinoma with general metastasis, and there are cases of carcinoma spreading to the tubes by direct extension, but this case shows no connection between the original tumor and the metastases.

The point of greatest interest in the case is the method of occurrence of the metastases, whether by the blood or lymph stream or by creeping up of the cells from below, or possibly by a multicentric origin of the tumor. There is no evidence of extension by the lymph or blood stream in this case, nor is it easy to imagine that if that were the method of origin, the metastases should occur in the epithelium of the tubal mucosa without previous involvement of some lymphatic structure.

The slides bring out two points bearing on this question. The first is that there is a general inflammatory reaction in the entire genital mucosa which suggests the possibility that a chemical or other irritant may have caused a multicentric development. The second point is that the lumina of the fundal glands and tubal mucosa show groups of very large desquamated cells which look like carcinoma cells, and it is possible that these represent wandering cells which have embedded themselves higher up in the tubal mucosa. The cervical glands of this case show a picture which might well represent appositional growth, since there are glands one half of which present perfectly regular epithelium, while on the other side of the lumen the cells have every appearance of the carcinoma which is directly adjacent to them. This idea of appositional transformation has been generally discarded for the unicentric idea of carcinomatous growth. Schöttlander still holds to the appositional transformation. Ribbert, Borst and others hold to the unicentric origin, while v. Hansemann and Lubarsch think that both methods of origin are possible.

If carcinoma is always a definite entity there can be no such thing as appositional growth, that is, a transformation of cells at the border into carcinoma cells; but, the general belief is that carcinoma is not such a fixed entity, as indicated by the transitions between carcinoma and benign growths, as adenoma, and also by the transitions between carcinoma and the so-called precancerous changes, so often seen in the breast. It is these unexplained appearances which are the support of the appositional transformation idea.

AN INTRACUTANEOUS METHOD FOR TESTING THE VIRULENCE OF DIPHTHERIA BACILLI

ABRAHAM ZINGHER, M.D., AND DAVID SOLETSKY, B.S.

(From the Research Laboratory, Department of Health, New York City)

The testing of the virulence of isolated strains of diphtheria and diphtheria-like bacilli is important for a number of reasons, the following being probably the most important:

1. It has been found that 4–8 per cent. of individuals in localities where diphtheria is endemic are carriers of bacilli morphologically and culturally like the diphtheria bacillus, but non-virulent in 30–50 per cent. of the cases. At the Willard Parker Hospital, Wilcox and Taylor found that 4.5 per cent. of the cases admitted to the scarlet fever wards were bacillus carriers, and of the isolated organisms only one half were virulent.

2. According to Neisser individuals who become persistent carriers after an attack of diphtheria show only non-virulent forms in fully 20 per cent. of cases. Such individuals could be discharged from quarantine if this fact were established.

Morphologically, the non-virulent strains cannot be separated from the virulent, while in their sugar fermentation reactions the two types are in a majority of instances similar. Hence, the animal test must be employed for final diagnosis in doubtful cases. The method so far in vogue for testing the virulence of diphtheria bacilli has been to isolate the strain, grow it for 48 hours in ascitic broth (1 part ascitic fluid + 2 parts veal broth), and inject 1 c.c. of the broth culture subcutaneously into a guinea-pig. A control pig is injected with the same amount of the broth culture, and also a small quantity of antitoxin (0.5 c.c. of a 100 or 200-unit antitoxin, which cannot be used for other purposes). If the strain is a true diphtheria organism, the control pig will live, while the test pig will die in 2–3 days, and the autopsy will show the typical lesions of death from the effects of diphtheria toxin; that is, subcutaneous œdema at the site of injection, often extensive, congested and hemorrhagic adrenals, fluid in the pleural cavities, congestion of the lungs with areas of partial consolidation.

This method is reliable, but an autopsy should be performed in all cases, in order to exclude death from other causes.

Recently M. Neisser has suggested that the virulence of cultures be tested in the following way: One loopful of a 24-hour Loeffler slant of the organism is suspended in 1 cc., 10 c.c., and 100 c.c. of physiological salt solution, and 0.1 c.c. of each suspension is injected intracutaneously on the abdominal surface of a guinea-pig. As a control some antitoxin containing 8 units per c.c. is added to an equal volume of the heaviest suspension, and 0.1 c.c. of the mixture is injected intracutaneously in the same guinea-pig. True virulent diphtheria bacilli will cause a local inflammatory lesion, with superficial necrosis, in 48–72 hours, the intensity of the reaction depending upon the number of injected organisms and their virulence. The skin at the site of the control injection should remain normal in appearance.

This method is analogous to that of Römer for the determination of small amounts of diphtheria antitoxin in sera. This consists of the intracutaneous injection of varying mixtures of the unknown antitoxic serum and a standard toxin; a slight excess of toxin produces a local necrosing lesion, while a neutral or over-neutralized mixture shows no effect on the tissues at the site of injection.

The method recommended by Neisser is fairly satisfactory, but, following his directions, we have occasionally noted that the direct addition of antitoxin to the bacteria in the control injection immunized the animals sufficiently to affect the test lesions to a considerable degree. If the amount of antitoxin added is diminished to avoid this general immunization, the local action of the bacteria is not completely inhibited, and lesions are found in both test and control areas.

For this reason the following modification of Neisser's method which has been found to be both reliable and economical is suggested.

Method.—Two guinea-pigs are used for the testing of from

4 to 6 different strains. One pig serves as a control and receives 0.5 c.c. of antitoxin (about 200 units per c.c.) intracardially at the time of making the tests, or intraperitoneally 24 hours before. The intracardial injection is the better, as it produces a complete inhibition of the local action of virulent bacteria in the control injections. The hair on the abdominal surface of each pig is removed, either by the application of a paste made of barium hydrosulphide, or preferably by simply pulling the hair out. This can be easily done, with less pain probably than is associated with the prolonged irritation following at times the application of the depilatory.

For the bacterial emulsion, a fresh 24 hour growth from an ordinary Loeffler slant is suspended in 25 to 30 c.c. of normal saline. It is important that the growth be not more than 24 hours old, since many of the bacteria die if the culture is kept for 48 hours or longer in the thermostat. Ice-box preservation of grown cultures also kills many of the organisms. Loeffler slants are used similar to those furnished by the New York City Department of Health for the purpose of diphtheria diagnosis. They should have a fairly uniform surface size and be sealed with paraffin to prevent drying of the medium. It is also important that the medium be not too acid, since excess of acid is apt to inhibit growth to a considerable degree.

Suspensions of the cultures to be tested are prepared in the above way, and 0.1 c.c. of each is injected intracutaneously into both pigs. For the purposes of injection the abdominal surface is divided into 4 to 6 areas, depending upon the size of the guinea-pig, and the injections are made as far apart as possible in order to avoid a fusion of the lesions. Four strains can be tested out on a medium-sized guinea-pig, and six on a larger one, without the danger of overlapping. A .5 c.c. or 1 c.c. Record syringe with a very fine steel (Burroughs Wellcome & Co. No. 3) or platinum iridium needle (Burroughs Wellcome Co. No. 22) is suitable for the injection. If the injections have been made properly a circumscribed elevation appears, which persists for 1 to 2 minutes.

The results of the tests are noted in 24 to 48 hours. Virulent strains produce a definite local inflammatory lesion, which shows a superficial necrosis in 48–72 hours. In the control pig the skin remains normal, when the injections are accurately carried out. With non-virulent strains, no lesion will be found in either control or test animal.

In this way, four cultures can be tested on two animals, as compared with eight animals necessary in the older way. By the use of large pigs, on whom six tests can be made, 10 out of 12 pigs can be saved, an advantage which is considerable when a large number of strains are to be tested.

We have tested by this method 20 non-virulent and 40 virulent strains of diphtheria bacilli, and the results obtained corresponded exactly with those obtained with the subcutaneous test for virulence.

A little practise is required for the proper performance of the injection; but with good technique, fresh Loeffler slants, and a proper 24-hour surface growth, the results will be found to correspond exactly with those obtained in the usual and less economical way for determining the virulence of diphtheria and diphtheria-like organisms.

We wish to thank Dr. Wm. H. Park, Director of the Research Laboratory, for many helpful suggestions in carrying on the work.

Discussion:

Dr. Hopkins: I would like to ask if necrosis could be produced by any other organism of this group, and were tests made to determine the specific action of the organism by using antitoxin as control.

Dr. Zingher: The local action of the diphtheria bacillus and of its toxin is very characteristic, and no lesions were produced when the *Bac. Xerosis* or *Hoffmanni* and about twenty non-virulent strains of diphtheria-like bacilli were tested by the intracutaneous test on the pig. The action of antitoxin injected intracardially into the control animal was found absolutely specific in counteracting the action of the virulent diphtheria bacilli.

Dr. Lambert: When the animals die, are the lesions in the adrenals absolutely characteristic; can they be mistaken for anything else?

Dr. Zingher: The congested and hemorrhagic adrenals in the presence of the other typical lesions are considered characteristic of the action of the soluble toxin of the diphtheria bacillus.

Dr. Warren: What do you do with the guinea-pigs afterwards?

DR. ZINGHER: We keep them for one or two months and then use them for complement.

DR. JOHNSON: Was there any fluid around the pericardium?

DR. ZINGHER: In the test animals, the total amount of bacteria injected represents as a rule, a sublethal dose. We have found occasionally in the regular test of virulence a small amount of fluid in the pericardium.

DR. JOHNSON: Did you test the blood and pleural fluid to find the organism?

DR. ZINGHER: We have found bacilli once or twice in several examinations of the heart's blood post-mortem. According to Wright 5 per cent. of the animals show the presence of bacilli in the heart's blood. The pleural fluid was not tested.

DR. ZINSSER: I am surprised at the large number of non-virulent strains. I ran through eighty and found only a few non-virulent. They all fermented like true diphtheria. Is the absence of virulence in carriers only temporary, has this been tested, and can these strains be brought to virulence?

DR. ZINGHER: Among scarlet fever patients admitted during the past year to the Willard Parker Hospital, Wilcox and Taylor found that about one half of carriers showed non-virulent organisms. In those who became carriers in the wards after admission fully 95 per cent. of the strains were found virulent. The absence of virulence in the organisms in the carriers harboring non-virulent bacilli is probably permanent. Roux and Yersin are of the opinion that both types are present in cases of diphtheria, the preponderance of each strain, however, varying with the severity of the disease; but that during convalescence all virulent strains undergo a definite diminution in virulence. Graham-Smith, Loeffler, and Park and Williams believe that the individual virulence or non-virulence is permanent with the strain. Dr. Williams following the methods advocated by several who claim they made this transformation failed to obtain the change. We are attempting at present to change a non-virulent into a virulent strain, according to the method suggested by Thiele and Embleton for the *Bac. Hoffmani.* They claim to have changed such a strain to a virulent diphtheria bacillus.

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DR. HANS ZINSSER, *President*

AGGLUTINATION OF TREPONEMA PALLIDUM

HANS ZINSSER, M.D., AND J. G. HOPKINS, M.D.

We wish to report some recent experiments which demonstrate definitely the presence of immune bodies in animals immunized with cultures of treponema pallidum. The earliest attempts to demonstrate antibodies in syphilis were in general unsuccessful. Failure to obtain immune reactions was due doubtless in a large part to the fact that the earlier workers did not have cultures of the organisms to work with and that the suspensions of organisms obtained from syphilitic lesions were too poor in treponemata to make success possible.

Noguchi in 1912 obtained positive complement fixation reactions in the sera of patients with advanced syphilis but not in those of inoculated rabbits, when he used as antigen aqueous extracts of syphilis cultures or extracts obtained from rabbit lesions. Kolmer, Williams, and Laughbaugh soon afterward reported similar results, using extracts of concentrated cultures. Positive results were given by a considerable number of sera from cases of human syphilis, but much stronger reactions with the sera of rabbits treated with treponema cultures.

In the course of our work with the treponema we have immunized a number of rabbits with suspensions of the organism obtained by centrifuging fluid cultures, and in this way have obtained suspensions containing enormous numbers of treponemata. The suspensions before injection were killed by heating one half hour at 56° C. In testing the sera of these immunized animals against similar unheated treponema suspensions we have observed powerful agglutination, which was observable both macroscopically and under the dark field, one of our rabbits giving very marked agglutination in a dilution of 1 : 2000. We have observed the agglutination, both when using a suspension of our own Strain "A" against which the animals were immunized, and also with a suspension of N-1, a strain isolated by Dr. Noguchi. The sera of some normal rabbits also showed power to agglutinate the treponema pallidum, but we have not so far obtained this reaction in a dilution greater than 1 : 20.

EXPERIMENT I. (MACROSCOPIC)

Suspension Strain "A."

Serum Rabbit "609" (intravenously treated with five injections of "A" suspension ranging from 1 c.c. to 5 c.c.).

Each tube contained 0.5 c.c. of serum dilution and 0.25 c.c. of suspension.

	Serum 609. First Bleeding		Serum 609. Second Bleeding		Normal Rabbit Serum	
	2 hrs.	15 hrs.	2 hrs.	15 hrs.	2 hrs.	15 hrs.
Conc.	+++	+++	+++	+++	+++	+++
1:2	+++	+++	+++	+++	+++	+++
1:5	+++	+++	+++	+++	+++	+++
1:10	+++	+++	+++	+++	+++	+++
1:20	+++	+++	+++	+++	±	±
1:50	+++	+++	+++	+++	0	0
1:100	+++	+++	+++	+++	0	0
1:200	+++	+++	+++	+++	0	0
1:500	++	+++	+++	+++	0	0
1:1,000	±	0	Not Set Up		0	0
1:2,000	0	0			0	0
1:4,000	0	0			0	0
Salt Sol.	0	0	0	0		

We have not yet proceeded far enough with our experiments to determine whether these reactions can be obtained with the sera of syphilitic patients or with those of rabbits showing syphilitic lesions.

We submit the preceding protocol as an example of the macroscopic tests made with the sera of immunized and of normal animals.

A CASE OF MULTIPLE MYELOMA

W. G. MACCALLUM, M.D.

Clinical History.—B. C., a woman aged 53, was admitted to the Presbyterian Hospital December 5, 1915, complaining of stiffness of neck and shoulders. This appeared gradually during the past three months but was not associated with any pain. On admission neck was held very stiffly, apparently entirely on account of spinal rigidity, as the muscles were not tense. There was a deep groove in the ribs running parallel with the sternum just outside the costo-sternal junction. The ribs were easily moved or bent and on very slight pressure gave the impression of being crushed. Thoracic and abdominal organs showed nothing abnormal. The spine was held rigid throughout. Both femora and tibiæ showed outward and forward bowing. No tenderness anywhere.

Blood count, examination.—

Leucocytes	9,000
Polymorphonuclears	54 per cent.
Lymphocytes	41 per cent.
Hæmoglobin	80 per cent.

On December 18 she developed signs of pneumonia and died five days later. The urine showed a moderate amount of albumen with hyaline casts but no Bence Jones protein.

X-ray photographs showed atrophy of the cervical vertebræ with destruction and collapse of the third, fourth and fifth. Areas of rarefication were thus demonstrated in many of the other bones including especially the ribs and long bones.

At the *autopsy* there was found a widespread confluent lobular pneumonia. The alterations of especial interest concerned the bones.

The ribs were rarefied and softened to such a degree that in most places they could be crushed easily between the fingers. They were especially thinned and greatly depressed on each side of the sternum and in these broad grooves nearly every one showed several fractures, often marked out by the growths of callus tissue.

The eleventh dorsal vertebra had collapsed to a certain degree, so that there was a slight angular deformity at that point. Three of the cervical vertebral centra had collapsed in the same way, bending the neck forward into a position in which it had become somewhat rigid.

One humerus was cut open and its cortex proved to be not more than 1 mm. in thickness, while the large narrow cavity was devoid of any cancellous lamellæ and filled with a mottled grayish red opaque marrow.

If we may judge from the X-ray photographs the same rarefication had occurred in the other humerus and in the bones of both forearms, as well as the femora tibiæ and fibulæ. No fractures had occurred in those bones and there was no sign of a tumor growth at any place. Many diagnoses were ventured to explain the rarefication of the bones and the many fractures but even at the autopsy the mere cellular character of the marrow exposed in the humerus did not suggest a tumor formation. The cut surfaces of the vertebræ were perfectly homogeneous and except for a slight opacity, not different from normal bones.

MICROSCOPIC FINDINGS

Smears from the marrow stained by Giemsa and Hastings' methods showed a remarkable uniformity of cells. Nearly all were rounded or oval cells of moderate size with rather deeply stained nucleus and practically no granules in the blue stained cytoplasms. There was found a pale halo around the nucleus. Occasional suggestions of basophilic granulations in small numbers were found.

Sections reveal a marrow composed in places of the normal cells in their usual arrangement, but nearly everywhere there were found compact masses of cells of one type pushing aside the normal marrow and extending everywhere into contact with the bone. Many fragments of cancellous lamellæ were found isolated in such masses. This tissue which was composed of cells corresponding with those found to be predominant in smears did not as a rule inpinge directly upon the bone but seemed always separated from it by a thin line of cells flattened almost like endothelial cells, which may have been osteoblasts. Nevertheless the effect in causing the destruction and wasting of the bone was extraordinary.

The cells which make up this tumor tissue were not like the myeloblasts described as forming the tissue in a case with numerous myelomatous tumors published in the *Journal of Experimental Medicine* some years ago (Vol. 6) but resembled more closely plasma cells. Whether they correspond exactly with plasma cells it is difficult to say, but after observing recently two cases in the laboratories of Dr. Norris and Dr. Warren, in which the whole character of the growth was very similar to this except in that definite tumors were found, it seems that two distinct types of myeloma must be recognized. Of these one is composed of myeloblasts, the other of cells practically identical with plasma cells. The explanation of the absence of albumoses in the urine is not clear but there have been recorded several other cases in which the urine was free from these bodies.

AORTIC ANEURYSM PERFORATING SUPERIOR VENA CAVA

W. G. MACCALLUM, M.D.

Clinical History.—H. G., woman, age 33, was admitted to hospital fifteen times between January, 1913, and her last admission, in the summer of 1914.

She had led a dissipated life with much abuse of alcohol and tobacco and very possibly had had syphilis. She entered the hospital complaining of shortness of breath and swelling of heart, face and neck. Ten days before admission in January, 1913, patient felt that her face was swollen; the same night she woke up with a choking sensation accompanied by pain. The neck was much swollen and the face bloated.

The physical examination showed œdema of the upper part of the chest and great dilatation of the capillaries in that region. A tracheal tug was felt. There were diastolic and systolic murmurs over the heart and a definite Corrigan pulse. The veins in the epigastric and hypochondriac regions were dilated and tortuous. Eye grounds normal.

During the next month the cyanosis and subcutaneous œdema in the upper part of the chest increased. There was a good deal of cough with rusty sputum.

In February of 1914 these conditions had become accentuated, marked dyspnœa and continuous murmur heard over the back as low as the fifth dorsal vertebra. There was a large accumulation of fluid in the right chest.

In May the superficial veins over the upper part of the chest were very prominent. Wassermann reaction positive.

June 16, 1914, admitted for the last time with still more exaggerated symptoms of the same character.

Death occurred on August 26.

At the *autopsy* the left lung was found to be a good deal compressed and to lie against the vertebral column. The heart was removed, together with the large vessels and the lungs and upon dissection it shows the following:

The heart itself is large, its walls somewhat thickened; the pulmonary artery is pushed forward by a saccular enlargement of the aorta beneath. This sac measures 5 to 6 cm. in diameter in each direction, so that the pulmonary artery is not really much compressed. The superior vena cava is delicate until it reaches the region of the auricle, where it becomes thickened and somewhat rigid and is adherent to the aortic dilatation. The thoracic portion of the aorta shows no dilatation or intimal change up to the end of the ascending arch. The large vessels of the arch pass off normally. They, like the aorta, are delicate and elastic.

On opening the heart and cutting through the aortic ring and the dilated aorta it is found that the left auricle is normal; the left ventricle dilated; mitral valves normal; its trabeculæ and papillary muscles flattened. Just beneath the aortic valves there are linear rough elevations which look like a beginning reaction to aortic insufficiency. The aortic valves themselves are fairly competent by the water test; their edges, however, are thickened and roll into a cord, so that insufficiency must have been possible, with dilatation, and probably existed all the time. Aortic orifice measures 7 cm. in circumference. The tricuspid and pulmonary valves are normal. Right ventricle is rather large; its walls measure 7 mm. in thickness; left ventricular wall measures 19 mm. in thickness. Coronary orifices are normal. Just above the coronory orifice there is a sudden dilatation of the aorta which ends as suddenly with the precipitous edge at the upper end of the ascending part of the arch. It is nowhere roughened or covered by thrombi, but the inner surface is very irregular and there are numerous small sacculations as if new small aneurysms started out from the wall of the old one. One of these, about 12 mm. in diameter, arises from the lower posterior part of the main aneurysmal sac and extends over toward the superior vena cava and presses against it; indeed, it practically pierces a way through the middle of the vein and divides it into two channels, fusing with the further wall of the vein, so that it looks like a bridge across the cavity of the vena cava, when it is exposed by opening that vessel. The blood was forced to pass on each side of this bridge and the two channels must have been still further narrowed by the thickening of the vein wall at this level. They now measure about 10–15 mm. in diameter. Under such circumstances there was no connection between the aortic lumen and that of the vein, since the aneurysmal saccule merely pushes its way through the middle of the vein to the opposite side; nevertheless it undoubtedly caused a good deal of obstruction to the return of blood from the head.

But the aneurysmal sac is found to have burst laterally as it passed across the vein, and a probe from the main aneurysm into the saccule emerges into the vein through a hole which proves to be in the wall of the bridge turned uppermost. This aperture is surrounded by a thrombus on the venous side and it was obviously through this that the blood rushed.

One receives the impression that this actual perforation may have taken place rather recently but the symptoms which it could have produced, such as murmurs and cyanosis, were already present to such a degree on account of obstruction of the vein and the valvular murmurs that it is surprising that the diagnosis could have been reached so accurately as it was.

PROGNOSIS IN PELLAGRA

A Preliminary Note

J. F. SILER, M.D., P. E. GARRISON, M.D., AND W. J. MACNEAL, M.D.

(From the Robert M. Thompson Pellagra Commission of the New York Post-Graduate Medical School and Hospital)

This is a preliminary report based upon the records of 1,162 cases of pellagra, all the cases which have been ascertained to have existed in Spartanburg County, South Carolina, up to October 15, 1914. We shall consider the prognosis first in regard to death rate in first year of the disease, or the year of initial attack, second in respect to liability of the survivors to have a recurrent attack in subsequent years or to escape further attacks, and third, the death rate in recurrent attacks. In each instance we shall inquire into the influence of race, sex and age upon the prognosis.

The gross death rate in the year of initial attack is shown in Table I.

TABLE I

Death Rate in Initial Attack of Pellagra

Year	Recorded Initial Attacks	Recorded Deaths in Year of Initial Attack	Indicated Death Rate, Per Cent.
Before 1908	56	13	23.2
1908	20	2	10.0
1909	57	15	26.3
1910	141	29	20.6
1911	229	32	14.0
1912	208	27	13.0
1913	242	37	15.3
1914	209	30	14.4
Total..........	1,162	185	15.9

The indicated death rate for the last four years is undoubtedly more accurate than for the earlier years, and we may say that in Spartanburg County about 14 per cent. of pellagrins die in the year of the onset of the disease.

Of the total 1,162 patients, no less than 977, or about 84 per cent., survived the year of the initial attack of pellagra, so far as these records go. Of these there were 179 cases originating in 1914, for whom the record of the first year is incomplete, and there were 101 patients who passed from observation. There remain, therefore, 697 pellagrins concerning whose subsequent histories we have some recorded data. In the aggregate, the records of these 697 patients show 1,037 instances of recurrence in subsequent years and 591 instances of freedom from recurrence, indicating roughly that those who survive one attack of pellagra have about one chance in three of escaping an attack of the disease in the following year. All cases are here considered together. Some have been followed for ten years, others for only one year; some have recurred every year, others have never shown any recurrence; others have passed one or two years in good health and have then suffered recurrent atacks, while others, again, have had one or more recurrences with subsequent freedom from the disease.

Recovery from pellagra is very difficult to recognize with certainty. Of the 711 recorded cases which originated previous to 1913, there are 91 instances of pellagra in the initial year with subsequent freedom from recognizable evidence of the disease for at least two years. These cases may, with some reservation, be designated as instances of recovery after the initial attack. The indicated rate for recovery after one attack is, therefore, 12.8 per cent. This indication must be taken with some reserve, however, because there were recorded 13 patients who again suffered an attack of pellagra after an interval of two years without recognizable evidence of the disease.

The gross death rate in the year of recurrent attack is shown in Table II.

TABLE II

Death Rate in Recurrent Attack of Pellagra

Year	Recorded Recurrent Attacks	Recorded Deaths in Year of Recurrence	Indicated Death Rate, Per Cent.
Before 1908	80	7	8.8
1908	22	2	9.1
1909	34	5	14.7
1910	58	7	12.1
1911	140	29	20.7
1912	226	32	14.2
1913	233	29	12.4
1914	244	19	7.8
Total..........	1,037	130	12.5

The death rate in recurrent attack is apparently somewhat less, but, on the whole, not significantly different from the death rate in the initial attack.

The correlation between race and prognosis in pellagra is indicated in Table III.

TABLE III

Relation of Race to Prognosis in Pellagra

	White Race	Negro Race
Recorded initial attacks	1,010	152
Recorded deaths in initial attack	121	64
Indicated death rate, per cent.	12.0	42.1
Recorded recurrent attacks	970	69
Recorded deaths in recurrent attack	107	23
Indicated death rate, per cent.	11.0	33.3
Recorded cases incident before 1913	630	81
Recoveries (after one attack)	84	7
Indicated recovery rate, per cent.	13.3	8.6
Recorded cases incident before 1912	450	53
Recurrence for three consecutive years	210	19
Frequency of chronic pellagra, per cent.	46.7	35.8

Those cases tabulated as recoveries were free from evidence of the disease for two years subsequent to the year of onset.

It would appear that the negro race in Spartanburg County has suffered much more severely from the acute type of pellagra, showing a much higher death rate, although as we have shown in previous papers, the negro race, as compared with the white race, is very much less frequently attacked by the disease in this county.

The correlation between sex and prognosis in pellagra is indicated in Table IV.

It would appear that the attack of pellagra is somewhat more decisive in males than in females, as the former show a higher death rate in both initial and recurrent attacks and also a higher recovery rate after the initial attack. Chronic recurrent pellagra, on the other hand, is relatively more common in females.

TABLE IV

Relation of Sex to Prognosis in Pellagra

	Females	Males
Recorded initial attacks[1]	816	345
Recorded deaths in initial attack	123	62
Indicated death rate, per cent.	15.1	18.0
Recorded recurrent attacks	757	282
Recorded deaths in recurrent attack	84	46
Indicated death rate, per cent.	11.1	16.3
Recorded cases incident before 1913	501	209
Recoveries after one attack	59	32
Indicated recovery rate, per cent.	11.8	15.3
Recorded cases incident before 1912	365	137
Recurrence for three consecutive years	175	54
Indicated frequency of chronic pellagra, per cent.	47.9	39.4

The correlation between age and prognosis in pellagra is indicated in Table V.

TABLE V

Relation of Age to Prognosis in Pellagra

Age	0–12	12–20	20–40	40–60	60 and Over
Recorded initial attacks[1]	207	86	533	250	67
Recorded deaths in initial attack	6	13	69	60	27
Indicated death rate, per cent.	2.9	15.1	12.9	24.0	40.3
Recorded recurrent attacks[2]	105	51	557	229	91
Recorded deaths in recurrent attack	4	6	60	43	15
Indicated death rate, per cent.	3.8	11.8	10.8	18.8	16.5
Recorded cases incident before 1913	97	61	327	171	42
Recoveries after one attack	31	12	34	13	0
Indicated recovery rate, per cent.	32.0	19.7	10.4	7.6	0.0
Recorded cases incident before 1912	49	44	239	130	31
Recurrence for 3 consecutive years	23	20	124	49	12
Relative frequency of chronic pellagra, per cent.	46.9	45.5	51.9	37.7	38.7

The comparatively good prognosis in children is clearly evident in the table.

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[1] One child has been omitted here because sex was not recorded.

[1] Nineteen cases omitted because age was not recorded.

[2] Four instances of recurrences omitted because age was not recorded.

Perhaps a few words should be said about insanity in pellagra. This topic has recently been discussed by Singer,[1] working in collaboration with us. He found mental disturbance in about 40 per cent. of cases of pellagra, chiefly symptomatic depressions and delirious states due to the intoxication of the disease, most commonly observed in men aged 21 to 40 and women aged 41 to 60. Such mental disturbance indicates a severe attack of the disease, but it clears up completely if the patient survives. Pellagra is much more common in individuals of faulty nervous organization, but chronic insanity or chronic nervous disease, due strictly to the pellagrous intoxication, if it occurs at all, is very uncommon.

AMŒBAS IN THE MOUTHS OF SCHOOL CHILDREN

ANNA WESSELS WILLIAMS, ANNA I. VON SHOLLY, AND CAROLINE ROSENBERG

(From the Bureau of Laboratories, New York City Health Department)

Since the announcement last summer by Smith and Barrett that an amœba genus, called *Endamœba* first by Joseph Leidy in 1879, is probably the cause of pyorrhœa alveolaris, and that an amœbicide, emetin, will cure the disease, interest has been stirred anew in the etiology and treatment of septic mouth conditions.

Among others, the Bureau of Laboratories of the New York City Health Department has begun a study of focal mouth infections helped by the interest and financial aid of Dr. Merritt, and this short paper is a first report of one phase of our study.

Soon after our observations were begun, we found that all cases presented to us by the several dentists who are collaborating with us, already had pus pockets formed in the teeth alveoli—that is, they were well-established cases—and when we began to inquire for beginning cases, we learned that the diagnosis of pyorrhœa alveolaris is made by most dentists—as is the diagnosis "trachoma" by most ophthalmologists—after the disease has made decided progress, so we decided to hunt for beginnings in school children.

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[1] Archives of Internal Medicine, 1915, XV, 121.

The school in which we had done most of our intensive trachoma work was chosen for starting our examinations. So far, about 475 children between the ages of 9 and 16 years have had a preliminary examination and, from 150 representative ones, stained smears have been made and examined for amœbas.

The 475 children have been divided roughly into those having healthy-looking gums and those having receding or spongy and bleeding gums. The healthy-looking gums were further divided into those with one or more decayed teeth, and those with apparently sound teeth. Needless to say, we found very few of the latter, that is, of those with apparently healthy gums and sound teeth—about 1 in 8. About one half showed unhealthy gums. The exact figures will be given later.

Results of Smear Examination of 150 Cases.—From most cases two smears were taken. The smears were made as follows: In the bad cases, the teeth and gums were cleaned with a cotton swab dipped in 50 per cent. alcohol; then with the flat end of a hardwood sterile toothpick, material was scraped from the margin of the gums and stroked lightly over a clean glass slide making 3 or 4 streaks. The slide was then fixed while still moist by dropping methyl alcohol on it, and it was ready to send to the laboratory. This was called the superficial smear. A second toothpick was used to get material from beneath the margin of the gums, from which another smear was made and fixed. This was called the deep smear. In the cases with clean teeth and tight healthy-looking gums, the first smear was made before cleaning with alcohol. All of these smears were stained with Giemsa's stain by A. G. Mann of our laboratory, and were examined by us for amœbas. In regard to the identification of amœbas by stained films, I would say that they are more certainly and easily identified by this method than by warm living preparations, though, of course, both methods should be used in studying all of the characteristics of these microorganisms.

Stained by Giemsa, the delicately but definitely reticulated cytoplasm of the amœba stains a clear blue, while the small nucleus shows rounded or irregular masses of red granules and threads, with often a central vacuole-like body. This nucleus without doubt corresponds to that most frequently described for *Entomœba histolytica,* but since we know that amœbas are notoriously variable in their entire morphology, according to their stage of development and to the conditions of growth, no definite opinion should be expressed as to the species of the forms found in these mouths until much further study has been made. The amœbas usually contain masses of undigested materials. They seem to take up very readily leucocytes, red blood cells, and many kinds of bacteria. I have placed a smear under the microscope from one of these cases.

Number and Percentage of Amœbas Found in Each Group of Children

I.	Healthy gums	+ 6 — 14	= 30 per cent. positive
II.	Healthy gums Decayed teeth	+ 11 — 11	= 50 per cent. positive
III.	Tartar and Receding gums	+ 37 — 10	= 84 per cent. positive
IV.	Spongy and Bleeding gums	+ 61 — 4	= 94 per cent. positive

+, positive; —, negative.

Of course, we can say nothing definite yet as to the significance of the amœbas in these mouths. Finding them so often in apparently healthy mouths and in such young children does not agree with the statements of Bass and Johns and of Barrett, that they are not found in healthy mouths. They base their statements on the fact that they were not able to demonstrate amœbas in healthy adult mouths. The demonstration by Le Wald before this society in 1907 of amœbas in the mouths of healthy individuals, cannot be discussed in this connection since the condition of the mouths and the ages of those examined were not stated. Moreover, we have so far made only one examination. Further investigation may show a larger percentage of amœbas constantly in the healthy mouths and a smaller percentage constantly in the unhealthy ones.

But these results as they stand are very striking, especially when we must add that most of the cases of spongy and bleeding gums show more amœbas in the films than the other cases. If we could only rule out other microorganisms the evidence for the pathogenic action of the amœbas would be strong.

We have, however, a marked opportunity to test the prophylactic effect of ipecac or its emetin alkaloid.

We propose to divide the cases showing amœbas into groups, one to be watched without treatment and others to be treated in different ways. We shall have a tooth wash made up containing a dilution of one of these drugs, and have the cleaning of the teeth done morning and afternoon in the school clinic under the supervision of the school nurse. We hope by combining these practical investigations with the laboratory studies that we may be able to help demonstrate the relationship of these amœbas to septic mouth infections.

Discussion:

Dr. Merritt: I had not the slightest idea of being asked to speak on this subject. I have for a long time been interested in the question of the etiology of pyorrhœa. We had very positive statements made in Philadelphia last July regarding the relation of amœbæ to the disease which has puzzled many practitioners. We thought we would get the research laboratory to find out how much truth there was in the matter. Bass and Johns have made such positive statements that it is important to know whether they are true or not. The indiscriminate use of emetin, too, ought to be checked.

I do not think that the etiology of pyorrhœa depends on endamœbæ. The numbers of amœbæ found, I do not think, bear any relation to the advance of pyorrhœa. The most advanced cases I have seen had the least and the most simple one, most. The more healthy mouths as a rule seem to have amœbæ. The fact that so many are found in the mouths of children is perhaps the best evidence that they have little to do with the etiology of pyorrhœa. Pyorrhœa is certainly not a children's disease. I have seen reports of cases in children, but personally have never seen the disease in a person under 18 years. Amœbæ are found not only in mouths not kept very clean, but also in mouths carefully kept.

Dr. Le Wald: Some years ago I found amœbæ in the mouth of a dentist extremely careful of his teeth. This discovery was made in the examination of the mouths of a large number of cases in the Philippines. The natives showed amœbæ in 75 per cent.; Americans, 71 per cent. In negative cases a repeated examination often resulted in positive findings. Thus I concluded that amœbæ were present in all mouths. Some of these people had carious teeth but not all. I concluded that amœbæ were constant inhabitants of the mouth. I have seen no reason to change that opinion.

Dr. Zinsser: In examining pyorrhœa cases we have found a great many spirochætes where the pus was taken from the gums. I think that is because of the presence of necrotic tissue, as in Vincent's angina.

DR. WILLIAMS: I should like to emphasize the question of the beginnings of the disease. Dr. Merritt says the earliest case he knows was 18 years. These children were from 9 to 16 years. Sixty-five of them had spongy, bleeding gums, slightly receding, with a great accumulation of leucocytes. The question is, are some of these beginning cases of pyorrhœa alveolaris?

DR. MERRITT: While there is usually a mild gingivitis in pyorrhœa, the disease affects essentially the alveolar bone. I, therefore, do not regard Dr. Williams' cases as true pyorrhœa. I think with proper care of the teeth pyorrhœa would never develop. One may have considerable loss of bone from the abrasive action of the tooth brush with no disease, or pyorrhœa with considerable loss of bone in addition to the gingival condition which accompanies it.

DR. KRUMWIEDE: I believe Dr. Williams found in these children as well as in pyorrhœa cases in adults enormous numbers of spirochætes with the amœba.

DR. WILLIAMS: Yes, in practically all mouths of these children except the most clean there were large numbers of spirochætes as well as many other varieties of microörganisms.

A BRILLIANT GREEN AGAR FOR THE ISOLATION OF TYPHOID BACILLI FROM FÆCES

CHARLES KRUMWIEDE, JR., M.D.

(*From the Division of Laboratories, Department of Health, New York, N. Y.*)

During the last year we have accumulated a great deal of data concerning the action of brilliant green on the growth of members of the typhoid-paratyphoid-colon group, when the dye is used in fluid and solid media. We hope to report the data shortly. At this time, I shall only describe a new plating medium for the isolation of the typhoid bacillus from stools.

The basis for the medium is an agar consisting of water, 1,000 c.c., Liebig's extract of beef, 3 grams, peptone, 10 grams, salt, 5 grams, and agar 15 grams. As this agar is used also as the basis for Kendall's modification of the Endo medium, we have found it most convenient to have the reaction made slightly alkaline to litmus, and to keep the medium in bottles of 100 c.c. amounts. To 100 c.c. of the melted agar is added 1 c.c. of the Andrade indicator (consisting of 100 c.c. of a 0.5 per cent. watery solution of acid fuchsin decolorized by the addition of 16 c.c. of normal soda solution). The indicator keeps well and is not affected by light. Normal acid is then added till the agar is distinctly red. The correct point is reached when the hot agar is red but the color disappears on cooling. Having determined the correct amount for one bottle, this is noted and a similar correction made for the bottles of this batch of agar as used. This method has the advantage over setting the reaction of the agar in bulk in that variations are possible, should they seem necessary.

One per cent. of lactose and 0.1 per cent. of glucose are then added, using sterile 25 per cent. solutions of the sugars. After mixing, the brilliant green is added. We are now using two concentrations of the dye. To one bottle is added 0.2 c.c. of a 0.1 per cent. solution, giving a final dilution of 1 : 500,000, and to another is added 0.3 c.c., giving a dilution of 1 : 330,000. Each bottle is sufficient for six plates, as the layer of agar must not be too thin. The plates are left uncovered until the agar is hard, and then covered with porous tops. The medium is inoculated by surface streaking.

On this medium, the typhoid colony is very characteristic. With the light passing obliquely through the plate, they have a peculiar snow-flake appearance. Using a hand-lens with artificial light, they have the texture of a coarse wool fabric.

The brilliant green inhibits the growth of many of the bacteria in the feces, and in this way gives a relative enrichment of the typhoid bacilli, if present. The degree of inhibition of the fecal types depends on the flora and varies widely.

Two dilutions of the dye are used to obtain the maximum inhibition of the fecal flora and still insure good growth of the typhoid bacilli, which vary somewhat in their reaction to the dye. Endo plates should be planted as well. Although we have never failed to isolate typhoid bacilli from the green dye agar when they were present on the Endo medium, a much greater experience is necessary before we can be sure that some strains of typhoid may not be exceptionally sensitive to the dye and therefore fail to grow. Any change in the constituents of the medium may upset the balance, and require a readjustment of the amount of necessary dye.

Testing the medium by adding traces of carrier stools to normal stools, we obtained the following results. Of 130 such mixtures, 71 gave negative results on Endo. Of these, 48, or 67 per cent., were positive on the green dye agar containing 1 : 500,000 of the dye. In another series of 28 stools, both strengths of dye were used. Of these stools, 18 were negative and 10 positive on Endo; 9 were negative and 19 positive on dye agar containing 0.2 c.c. and 3 were negative and 25 positive on dye agar containing 0.3 c.c. One gave negative results with dye agar containing 0.3 c.c. due to almost complete inhibition of growth, but was positive on dye agar containing 0.2 c.c. Nine stools obtained for diagnosis during a localized epidemic gave similar results. All were negative on Endo, 4 were positive on the dye agar containing 0.2 c.c. and 5 on the dye agar containing 0.3 c.c.

Discussion:

Dr. Zinsser: In the preparation of typhoid media one difficulty we have found is the uniformity of production of media. In the green media is there any difficulty in getting uniform preparations? Is the dye constant?

Dr. Krumwiede: We have used the preparation of one manufacturer. Brilliant green, extra crystals, No. 366, Bayer. It is more uniform than the malachite green.

Dr. Zinsser: How is the media titrated?

Dr. Krumwiede: We have made the medium alkaline to litmus. The Andrade indicator gives a red color in hot media and is colorless when cold, when the correct point is reached; as a rule, 5 normal Hcl. is required to bring it to that point when originally alkaline to litmus.

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DR. HANS ZINSSER, *President*

THE ETIOLOGY OF TYPHUS EXANTHEMATICUS[1]

I. BACTERIOLOGICAL STUDIES

HARRY PLOTZ, M.D.

Abstract[2]

In a preliminary communication published in the *Journal of the American Medical Association,* May 16, 1914, I described an organism which I considered to be the etiological agent in typhus exanthematicus. The organism, a bacillus, was recovered from the blood of five cases of European epidemic typhus fever and of six cases of the mild endemic form of the disease known in the

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[1] From the Pathological Laboratory, Mount Sinai Hospital, New York. For the financial support of these investigations, we are indebted to two contributors who desire to remain anonymous.

[2] A complete report will be published shortly in *The Journal of Infectious Diseases.*

United States as Brill's disease.[3] Since that time I have had the opportunity of studying a much larger series of cases with the same cultural methods, and the results, together with the serological and experimental studies, have confirmed the opinion then expressed. At the present time, we shall give only those data which bear upon the question of etiology, leaving other phases of the work for future reports.

The findings of previous investigators will not be discussed because none corresponds to the organism which I have found.

The differentiation of the mild endemic form of typhus fever seen in New York from other fevers, especially short term typhoids, is due to the clinical insight of Dr. Nathan E. Brill. Its similarity to typhus fever was later noted by Louria and G. A. Friedman. Following this came the striking cross immunity experiments of Anderson and Goldberger, which demonstrated that the disease described by Brill was most likely a mild form of typhus fever.

My studies were begun with the endemic form of the disease, using the viewpoints that had been established regarding typhus fever.

It had been demonstrated by early experiments that the virus exists in the circulating blood during the febrile period of the disease. It was also evident from previous studies that the virus was not filterable. Ricketts and Wilder searched the blood microscopically and saw a bacillus which they described. There is no evidence that the bacillus seen by them is the same as that which forms the basis of this report. Fried and Sophian could find no organisms in smears of blood made from five cases of endemic typhus fever.

It was surmised that because typhus fever had been proved to be an insect-borne disease, a protozoan origin would needs be found, but Ricketts and Wilder, in refutation, pointed out that the organism could be bacterial.

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[3] As these studies give the definite proof that the cases of European typhus fever and the cases of "Brill's disease" are due to the same organism, we shall designate the European cases, *epidemic* typhus fever, and the New York cases, *endemic* typhus fever.

Aerobic cultures of the blood had been made by a number of observers, but as these had all been negative, I decided to use anaerobic methods.

Up to the present time, eleven cases of European epidemic typhus fever and forty cases of the local endemic form of the disease have been studied. I am indebted to Dr. Joseph J. O'Connell, Health Officer of the port of New York, for the privilege of studying the epidemic typhus cases and to the attending physicians of Mount Sinai Hospital for studying thirty-six of the forty endemic cases.

This work, as well as the investigations which form the basis of the subsequent papers, was carried out under the direction and with the helpful assistance of Dr. E. Libman. It was the energy and stimulus which he supplied to the work which helped us to carry it to a successful conclusion.

We wish also to thank Dr. F. S. Mandlebaum, pathologist of the hospital, for many courtesies and for placing all the facilities of the laboratory at our disposal.

METHODS OF CULTURE

Aerobic Methods.—Fifteen cubic centimeters of blood are withdrawn and inoculated into flasks of plain bouillon and glucose bouillon and tubes of agar and glucose agar, plates being made from the latter.

Because of the report made by Rabinowitch that he had obtained positive blood cultures in cases of typhus fever by the use of an aerobic method, his medium was tested. The results were negative in all the cases tested although some of these cases gave positive results with the anaerobic method.

Anaerobic Methods.—The first successful results were obtained in two cases of endemic, and one case of epidemic typhus fever by the use of the ascitic fluid-kidney tissue medium, layered with liquid petrolatum, as advocated by Dr. Noguchi for the cultivation of spirochetae. I am indebted to Dr. Noguchi for a demonstration of his methods. The method introduced by Liborius-Veillon proved to be more satisfactory,—modified in that serum-glucose-agar, originally suggested by Libman as an optimum medium, was used instead of glucose agar.[1]

Serum Employed.—The sterility of the fluid must be ascertained before use, by thorough aerobic and anaerobic methods, such as will be described in the full publication. Studies must also be made to exclude hemoglobinophilic organisms.

DEVELOPMENT OF THE ORGANISM OBTAINED: SUBINOCULATIONS

Colonies of the organism appear in the tubes in from three to sixteen days. Negative tubes were observed twenty-one days. On development, the colonies are removed and inoculated on slants of one half per cent. glucose-serum-agar, the latter tubes then being inserted in Buchner tubes.

The colonies usually appear in the lower two or three centimeters of the tube, occasionally also higher, but never within three centimeters from the top of the medium. The colony first appears as a small opaque spot. As it grows larger a brownish area of precipitation[2] develops in the medium about it. By direct sunlight or incandescent electric light (frosted bulb) the colony itself appears round and opaque, varying in size from one to six millimeters in diameter (including the area of precipitation), the size of the colony depending on its stage of development. On cross section it is Y-shaped, brownish in appearance, and soft in consistency. The arms of the Y are fusiform.

MORPHOLOGY

The organism is a small pleomorphic Gram-positive bacillus, which is non-motile, non-encapsulated and not acid fast. Its length varies from 0.9 to 1.93 micra, its breadth being from one fifth to three fifths the length. Most of the organisms are straight, occasional ones are slightly curved. Coccoid forms also occur. The ends are rounded or slightly pointed. In smears from solid media, the organisms lie end to end, side to side or at angles to one another, there being no definite arrangement. Degeneration and involution forms appear early. The organism produces no spores. Polar bodies are occasionally demonstrable either at one end of the bacillus or both.

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[1] The details of the method will be described in the full publication.

[2] This phenomenon, which is due to acid production, was first described by Libman in 1901, when he advised the use of media containing carbohydrates and sera (non-coagulated) for the growth of poorly growing organisms.

CULTURAL CHARACTERISTICS

Besides the usual studies of cultures grown in Buchner tubes or in Novy jars, inoculations were made in a variety of media in long test tubes. A saline emulsion of the organism was inoculated in the following media:

Agar,
½ per cent. glucose agar,
2 per cent. glucose agar,
Sugar-free bouillon,
2 per cent. glucose bouillon.

These media were used alone; layered with liquid petrolatum, with the addition of kidney tissue; with ascitic fluid; with ascitic fluid and kidney tissue; and with ascitic fluid and kidney tissue layered with liquid petrolatum.

There was no growth in the agar or sugar-free bouillon after twenty days observation. Growth was present in one half per cent. glucose-agar with ascitic fluid and kidney tissue, but was better in two per cent. glucose-agar. There is a minute flocculent growth at the bottom of the glucose-bouillon tube after eight days.

In Buchner tubes, after three days, on glucose-serum-agar there is a creamy white growth which does not spread and which in older cultures may become light brown in color. There is moderate precipitation which increases after three days and increases on subculture.

On potato, after four days there is a practically invisible whitish growth demonstrable by scraping the medium. On gelatin, fifteen per cent., there is no growth even in the thermostat. On litmus milk, there is a slight amount of acidity after eighteen days. On Loeffler medium, there is very slight growth.

The organism from both the epidemic and endemic cases was found to produce acid and precipitation in glucose, maltose, galactose and inulin and not in raffinose, mannite, arabinose, saccharose, dextrin and lactose. The thermal death point was found to be 55° C. for ten minutes. The organism was found to be non-filterable.

On plates, colonies could be developed only in glucose-serum-agar, appearing after seven days. Surface colonies were obtained only once. The deep colonies varied in size from pinpoint to one millimeter. They were round, oval or triangular, the last type predominating. Microscopically, the colonies were triangular or acorn-shaped, sharply circumscribed with irregular contour.

Aerobic Studies.—The organism is an obligatory anaerobe. It was at first thought that slight growth had occurred aerobically, after anaerobic cultivation. The strains were inoculated on a variety of aerobic media, with negative results.

GENERAL RESULTS OF THE BLOOD CULTURE STUDIES

Blood cultures were taken in seven cases of epidemic typhus fever during the febrile stage and the bacillus isolated in each.[1] From an eighth case blood was inoculated intraperitoneally into two guinea-pigs which developed the typical febrile reaction and from the blood of which at the height of the reaction the organism was obtained.

Blood cultures were taken in thirty-four endemic cases up to the time of crisis. Eighteen of these, or fifty-three per cent., yielded the bacillus.

Blood cultures were taken after the crisis in six of the cases of epidemic typhus fever and all proved negative. In three of these the organisms had been recovered during the febrile period of the disease. Nine of the endemic cases were cultured after the crisis and the bacillus was found in one of them twelve, and in the other, thirty-six hours after the crisis.

With the identical technic used for isolating the bacillus in typhus fever, anaerobic blood cultures were taken on one hundred and ninety-eight control cases and in no instance was an organism recovered which in any way resembled the bacillus recovered from cases of typhus fever.

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[1] In the full paper these facts will be elucidated by tables giving all the details.

THE IDENTITY OF THE ORGANISMS ISOLATED FROM VARIOUS SOURCES

The organisms isolated from the endemic typhus cases were found to be culturally identical with those isolated from the epidemic typhus cases. Not the slightest variation was found. The organisms isolated from guinea-pigs and monkeys in which the disease had been produced experimentally were also exactly the same.

A careful study of the facts in the tables brought out the interesting observation that the organism occurs more abundantly in the blood on the fourth and fifth days before the crisis than later. It was also found that the bacillus was found eighteen times as abundantly in the blood of the epidemic as in the blood of the endemic cases. Without laying too much stress on this exact figure, it is very evident that the bacteriemia is more intense in the epidemic than in the endemic cases. The two cases of epidemic typhus fever which had the most marked bacteriemia proved fatal.

Because of the late maturation of the colonies, the results of the blood cultures are usually only known after the termination of the illness and therefore can be used only for confirming a previously made diagnosis, but in two instances of supposed influenza, the organism was found after the crisis and then a careful investigation revealed the fact that both of the individuals had passed through an attack of typical typhus fever.

CONCLUSIONS

Attention has been drawn to the fact that the virus of typhus fever circulates in the blood of an infected person or animal, that the virus is not filterable and, therefore, of microscopic size; and that aerobic cultures made with a great variety of media are negative. Hence it was considered plausible that the infective agent is an anaerobic organism. By means of an anaerobic method of blood culture a bacillus was isolated in pure culture from seven cases of European epidemic typhus fever, or one hundred per cent. of the cases studied during the febrile period. With the same method the identical organism was recovered during the febrile period of the local endemic form of the disease, in eighteen of thirty-four cases, or fifty-three per cent., and in two additional cases taken after the crisis. Similar blood cultures made in one hundred and ninety-eight control cases yielded no such organism.

Considered in conjunction with the serological and experimental evidence which follows, these facts prove that this bacillus is the etiological factor in typhus exanthematicus.

Following a very kind suggestion of Professor William H. Welch, it has been deemed advisable to name the organism, *Bacillus typhi-exanthematici*.[1]

THE ETIOLOGY OF TYPHUS EXANTHEMATICUS

II. SEROLOGICAL STUDIES[1]

PETER K. OLITSKY, M.D.

Abstract[2]

I. COMPLEMENT FIXATION DURING THE COURSE OF TYPHUS FEVER

In the beginning three antigens were used, one made up of the epidemic typhus organisms, one of the organisms of endemic cases, and a third of a mixture of both organisms. Later it was found better to use an antigen made up of both strains of the typhus bacillus. The results were then found to be more uniform, and positive reactions more common.

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[1] The name *Bacillus typhi exanthematici* was applied by Klebs in 1881 to a hypothetical organism in typhus fever. The name was never actually used, as the search for the organism proved futile. The hyphenating of "typhi" and "exanthematici" succeeds in giving us a binomial designation properly descriptive.

[1] This work was done under the tenure of the Eugene Meyer, Jr., Fellowship.

[2] A complete report will be published shortly in *The Journal of Infectious Diseases*.

Eleven cases of epidemic and thirty-four of endemic typhus fever were tested. Of twenty-five cases studied at the height of the disease, only two were positive, one giving a ++ reaction, the other a ++++ reaction. Both were tested one day before the crisis. The latter had a negative, the former a positive blood culture. Ten cases were tested at the crisis. Of these, two gave ++++ reactions, one a +++, one a ++, and the rest were negative. In the post critical stage of the disease, nine epidemic and thirty endemic cases were studied. Of these, 71.8 per cent. gave positive reactions.

The complement fixing antibodies reach their maximum concentration between the second and twelfth day after the crisis. Their persistence was studied in two cases, in one of which the reaction became negative between the sixty-fourth and the one hundred and thirteenth day after the crisis, and in the other case between the third and thirty-ninth day after the crisis. One may trace the development in an individual case of a negative reaction at the height of the disease to a ++++ reaction after the crisis.

One hundred and four control cases were studied with negative results.

Complement Fixation on Typhus Fever Cases with other than Typhus Antigens.—These antigens were made of *Bacterium coli, Staphylococcus aureus, gonococcus, streptococcus* (single antigens made of various types) and some Gram-positive anaerobic bacilli, having a morphology similar to that of the typhus bacillus (*Bacillus acnes* and bacilli isolated from ascitic fluid and from rabbit's kidney). These antigens were prepared in the same manner as the typhus antigen.

2. AGGLUTINATION REACTIONS DURING THE COURSE OF TYPHUS FEVER

Microscopical reactions were found to be better than macroscopical. Comparative experiments demonstrated that an epidemic typhus agglutinogen gave clumping with serum from endemic as well as from epidemic typhus fever, and an endemic typhus agglutinogen likewise gave positive agglutination with serum from patients suffering from either form of the disease.

In applying a mixed agglutinogen, positive agglutinations were obtained in more instances than positive complement fixation tests. For reasons which we will give later, only reactions in dilutions of 1 to 50 or higher were considered positive.

In forty-three cases of typhus fever during some period in the disease, thirty-nine, or 90.8 per cent., were found positive. Of twenty-four cases tested all were negative, except two which were tested a day before the crisis; both had a titer of 1 to 100. Of ten cases tested on the day of crisis, three were positive and seven were negative. In the apyrexial period, thirty-eight cases were studied of which only three were negative and 92.1 per cent. positive. The agglutinins evidently follow a curve similar to that of the complement fixing antibodies, the maximum concentration developing about four days after the crisis. The agglutinins have been demonstrated as late as five months after the crisis.

Agglutination Tests on Control Cases.—There were ninety-six control cases studied at various periods. Seven of these gave an agglutination in dilution of 1 to 20, but not in high dilutions. Of the seven cases one was a brain tumor; two, valvular defects; two, general paresis; one, carcinoma of the tonsil; and one, a case of alcoholism. The complement fixation in all these cases gave a negative result. It is because of the results in these cases that we regarded only such results as positive that gave agglutination in dilutions of 1 to 50 or higher.

There were three control cases in which the occurrence of a previous attack of typhus fever could not be excluded, in which reactions were found in dilutions of 1 to 100 to 1 to 200.

Agglutination with other than Typhus Organisms.—We endeavored to use as an agglutinogen all such bacteria as we were able to obtain in cases other than typhus fever, which showed any resemblance, morphologically, to the typhus bacillus, including the *Bacilus acnes*. In all such instances, when the agglutinogen was mixed with convalescent typhus serum, the results were negative.

3. PRECIPITATION REACTIONS IN TYPHUS FEVER

Of ten cases studied at the height of the disease all were negative. Of three cases tested at the crisis, one showed distinct clouding in 1 to 1,000 dilution. Fourteen, or 73.6 per cent. of nineteen cases tested in the fever-free period, gave precipitations. Three of these reacted in dilutions of 1 to 1,500; four in dilutions of 1 to 1,000, while the rest showed precipitates in dilutions of 1 to 100.

With each test a negative serum was included. Such control cases always gave negative reactions. The precipitin formation is evidently absent at the height of the disease but becomes in evidence at the crisis and proceeds to increase until well along in the post critical stage.

4. BACTERIOTROPINS

While discrepancies are common in making such studies, the average results demonstrate that the opsonic index increases at the crisis and remains high in the convalescent stage of the disease. Similar rises were noted in artificially immunized serum (rabbit). The probability is that this method is one of the most potent on the part of the patient in overcoming the infection. We noted during these examinations that phagocytized bacteria had a marked tendency to become fragmented and take the stain very lightly. Lysis of the typhus bacilli apparently is very active within polymorphonuclear cells of typhus immune subjects.

Other features of this work, the occurrence of allergic reactions, and the nature of the toxin produced, will be reported later.

5. THE IDENTITY OF THE ORGANISM ISOLATED FROM CASES OF ENDEMIC TYPHUS FEVER TO THAT ISOLATED FROM CASES OF EPIDEMIC TYPHUS FEVER

For the purpose of investigating this subject, cross fixation investigations were made both in artificially produced immune serum as well as in natural immune serum and similarly by cross agglutination tests. From studies of rabbit's immune sera the conclusion is definite that from a serological viewpoint the organism obtained from epidemic and the one from endemic typhus fever are two strains of the same bacterium.[1]

6. SEROLOGICAL STUDIES IN ANIMALS

Monkeys.—Of six monkeys that reacted to the typhus virus, complement fixation and agglutination tests were positive in five, or 83.3 per cent. These reactions were still positive twenty days after the crisis.

As the agglutination (Widal) reaction in typhoid fever is no absolute sign of protection from that disease, so in typhus fever, the serological manifestations of complement fixation and agglutination, while often appearing in connection with immunity, are no indications of immunity. The four monkeys mentioned above, two with reactions and two without, were injected with active typhus virus. Three of the monkeys reacted, one did not.[2]

We shall report in the future our results in prophylactic vaccination against typhus fever, a work which is now in progress.

Guinea pigs.—Although these animals were proved to be immune after reacting to typhus virus, the serum contained no agglutinin or complement fixing bodies. It is most probable that this animal develops its high grade of immunity by means of its tissue elements and only to a very slight degree by means of the circulating blood.

There is a possibility that bacteriotropins may exert a great influence in these animals and likewise add to the factors of the cellular immunity which may be present.

Rabbits.—Rabbits are not susceptible to the typhus bacillus in small amounts. When small yet increasing amounts of bacteria are given, they develop very potent immune serum.

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[1] See tables in full publication.

[2] The production of complement fixing bodies as well as agglutinins in monkeys can be paralleled in man.

CONCLUSIONS

A careful examination of the above results leads one to the belief that there is most intimate relationship between the typhus bacillus and typhus fever. All the serological reactions occurred in orderly manner, usually becoming demonstrable at the time of the crisis and increasing in concentration after the crisis. There is but one conclusion possible: Typhus fever is a reaction against the organism which has been isolated by Dr. Plotz. The presence of the reactions does not indicate absolute immunity.

As regards the relationship of the occurrence of the reactions to the results of the blood cultures, these facts are noted:

There were sixteen cases in which the blood culture was taken at the height of the disease and in which bacteria were not found. By examining the table which is included in the full publication, it will be seen that most of our strongest serological reactions occurred in just such cases.

THE ETIOLOGY OF TYPHUS EXANTHEMATICUS

III. EXPERIMENTAL STUDIES[1]

GEORGE BAEHR, M.D., HARRY PLOTZ, M.D., AND
PETER K. OLITSKY, M.D.

Abstract[2]

For the purpose of ascertaining whether the organism recovered from the blood of typhus fever patients was also found in animals having experimental typhus fever, a series of twenty-four guinea pigs was inoculated with defibrinated blood obtained from patients or animals with the disease. Blood for cultures was obtained by direct aspiration of the heart sometime after the

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[1] This work was done under the tenure of the Eugene Meyer, Jr., and Moses Heineman Fellowships in Pathology.

[2] A complete report will be published shortly in *The Journal of Infectious Diseases*.

onset of the fever. From eight, or thirty-three and one third per cent. of the guinea pigs in this series, the same bacillus was isolated from the blood as had been obtained from the blood of individuals with typhus fever. The percentage of positive blood cultures is less than that obtained in human cases because we were forced to use very small quantities of blood for the cultures, the average amount being three cubic centimeters.

In the positive blood cultures the colonies averaged about one per cubic centimeter of blood. This is more than was found in the patients with endemic typhus fever, but less than in individuals with epidemic typhus.

In only one of nine guinea pigs having mild febrile reactions was the blood culture positive. In fifteen animals with severe reactions the blood culture was positive in seven. This relationship between the blood culture results and the severity of the disease was also observed in human typhus cases.

A majority of the blood cultures were positive when taken between twenty-four and seventy-two hours after the onset of the fever. This observation assumes especial interest when the temperature curves of thirty-five other animals which were permitted to live for the entire course of their disease are analyzed. In the majority of instances (sixty-six per cent.) the fever reached its highest point on the second or third day of the disease, in other words between twenty-four to seventy-two hours after the onset. This is the period when the blood culture is most frequently positive in the infected animals.

Two strains of bacilli obtained from two of the epidemic typhus cases (epidemic cases 1 and 5) were inoculated intraperitoneally into guinea pigs. During the first twenty-four hours after the inoculation both animals developed a slight transient elevation in temperature, after which it remained normal for six and eighteen days respectively. The temperature then rose suddenly to 104° F. (40° C.) at which level it continued. One animal lived during the entire course of the disease, the fever lasting six days and ending abruptly. On the third day of the disease in the other guinea pig a blood culture was made. Seven days later a characteristic colony appeared in one of the culture tubes. This organism upon subculture proved to be both morphologically and culturally identical with *Bacillus typhi-exanthematici*.

Other guinea pigs and monkeys were subsequently inoculated with the two strains of bacilli used in the above experiments or with strains from three other epidemic cases after they had been on artificial media (glucose-serum-agar) for more than three or four weeks, and all strains were then found to have completely lost their virulence. In each instance the inoculation was followed by only a slight transitory rise in temperature during the first thirty-six hours, a reaction also observed in the experiments with virulent organisms and apparently due to a toxin action. After cultivation on artificial media for more than a month, all strains of epidemic organisms also lost this toxin action.

This rapid loss of virulence explains why the reproduction of typhus fever in animals by inoculation of the bacilli was not done more extensively. The positive blood cultures in cases of epidemic typhus fever were obtained at the very beginning of the work. Subsequently when the importance of inoculating the organisms into animals within the shortest possible time after isolation was realized, no more cases of epidemic typhus fever were available.

We hope to have the opportunity very shortly to amplify this phase of the work. It is important, however, that we have demonstrated that with bacilli recently isolated from epidemic cases of typhus fever the disease can be reproduced in animals and that at the height of disease so produced the identical organism can again be recovered from the blood.

Experiments carried out with six strains of bacilli from the endemic cases (endemic cases 1, 2, 18, 23, 27 and 40) and with those isolated from three animals (guinea pigs 21, 24 and 103) with typhus fever, revealed the fact that they lost their virulence outside the body still more rapidly than did the epidemic typhus strains. Before sufficient growth could be obtained for inoculation, the organisms were on artificial media for two to three weeks, or even longer. When finally ready for inoculation such organisms were not only avirulent, but they did not even possess the power of producing toxic rise in temperature after inoculation, a property only lost by the epidemic typhus organisms after cultivation for over a month.

Both morphologically and culturally the organisms isolated from the epidemic and the endemic type of the disease proved to be identical, and both were found to possess the same specific antigenic properties. This variation in virulence and in toxin production is the only essential difference which we have been able to demonstrate between the organisms derived from the two sources. Its importance lies in that it supplies us with a possible reason for the difference in the degree of bacteriemia and in the severity of the two diseases.

RELATION BETWEEN THE NUMBER OF BACILLI IN TYPHUS BLOOD TO ITS INFECTIVITY

The observations presented thus far in this paper have demonstrated the association of the bacillus recovered from cases of typhus fever with the disease as reproduced in the experimental animals. Further evidence from the experimental standpoint that this organism is the etiological agent in typhus fever was readily obtained by a study of the relation between the number of the bacilli in typhus blood and its infectivity.

A calculation of the average amount of organisms apparently contained in the quantity of blood inoculated into each animal was estimated from the total number of colonies which developed in the blood culture tubes.[1]

The uniformity of the observations on this series of fifty-one animals clearly indicates that typhus blood which contains no bacilli, or only very few bacilli, is *not* infective for animals. Typhus blood in which the bacilli are more numerous is infective. These experiments therefore demonstrated that infectivity is absolutely dependent upon the presence of a sufficient number of these bacilli. This in itself is conclusive evidence of their etiological significance.

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[1] The tabulation of these results and the protocols of the experiments upon which these conclusions are based will be found in the complete paper in *The Journal of Infectious Diseases.*

A study of the serum of individuals who have been exposed to infection by contact with human beings or animals suffering from typhus fever, led to the interesting observation that some of these people develop positive complement fixation and agglutination reactions without having had any clinical evidences of the disease.

CONCLUSIONS

It has been shown that a bacillus, identical with that recovered from patients with typhus fever, can also be isolated from the blood of animals in which the disease has been reproduced by inoculation of typhus blood. In such animals, the frequency of the bacilli in the blood is directly proportional to the severity of the illness. In individual animals it is greatest at the height of the disease.

It has also been shown that with the bacilli isolated from epidemic cases of typhus fever, it is possible to reproduce the disease in animals. And furthermore, at the height of the disease in such animals, the identical organism can be recovered from the circulating blood.

Finally, it has been shown that typhus blood is only infective if it contains a sufficient number of these bacilli.

From these observations and the results of the bacteriological and serological studies, we believe ourselves justified in concluding that this bacterium is the causative agent in typhus exanthematicus.

Discussion of papers by Dr. Plotz, Dr. Olitsky, and Drs. Baehr, Plotz and Olitsky:

Dr. Noguchi: I am very fortunate indeed to have the opportunity of hearing and discussing the important papers just presented by Drs. Plotz, Olitsky and Baehr this evening, as I had the great privilege of being shown the organism by Dr. Plotz when he first isolated it from typhus cases about a year ago. At that stage of the investigation it was of course too early to form any definite opinion as to the etiological relationship between the organism and the disease in question, but now that further evidences that are required for settling such a problem are brought out in a most strictly scientific and thorough manner, it must be evident to anyone that the causative organism of this mysterious disease has been at last discovered. I was interested to hear that not all ascitic media could be used for the first isolation of the typhus organism, as this agrees with my own experience with the cultivation of spirochætes, poliomyelitic microörganism, rabies virus, etc. The question of active and passive immunity, as well as the distribution of the organism within and without the body of the infected host, will undoubtedly be soon solved. I wish to congratulate Dr. Plotz, Dr. Olitsky and Dr. Baehr for this splendid contribution to the study of communicable diseases.

Dr. Park: When Dr. Plotz first announced his discovery many of us were somewhat skeptical, as it seemed an accident. But to-night it is plain that this investigation of the disease was carefully planned and the discovery was after much preliminary work. Dr. Noguchi's previous studies on anaerobic cultures led up to it. I see very few points that have been left unfinished in the work, but there are two points apparently not brought out which are fundamental. There may not have been time to take them up yet. Is the freshly isolated culture virulent for other animals than the guinea pig and monkey? I think Dr. Baehr said that in rabbits large amounts would cause infection, but are other animals similarly susceptible? Secondly, are the monkey and guinea pig, which have been infected by the fresh cultures and recovered from the attack, immune to the blood from a case of typhus fever in man or monkey at the height of the infection? If these two questions can be answered satisfactorily there is no other evidence required. The work appears to have been splendidly done.

Dr. N. E. Brill: These most brilliant papers afford me in particular the greatest satisfaction, because they settle in my mind that the etiology of typhus fever is at last discovered; because they offer for the first time the absolute proof that the disease described by me, which I was the first to assert might be an attenuated form of typhus fever and which I have always believed was related to typhus fever, ever since the important experimental studies on the cross immunity of these two types of fever, is due to the same organism as is epidemic typhus, and hence that they are identical as Anderson and Goldberger asserted; and because the infective agent just revealed may afford a rational means for prophylaxis and perhaps for the cure of typhus fever. We must accept from the work which has been communicated to us to-night that typhus fever exists clinically in two forms, one the classical, epidemic and virulent variety, and the other the mild, attenuated form to which I had the honor of calling the attention of physicians and which exists continually in New York City and in other cities of the United States. Up to this evening, while I had admitted the relationship of these two forms, I could not agree with the deductions of Anderson and Goldberger which they drew from their experimental work, viz., that their work proved the absolute identity of typhus fever and so-called Brill's disease. The reasons, which they urged justified their conclusion, were that when an animal was infected with Brill's disease it was subsequently immune to infection with typhus blood, and on the other hand that when an animal was infected with typhus fever it became immune to infection by the virus of Brill's disease. In the discussion of this feature of their work presented by me at the meeting of the International Medical Congress held in London in 1913, I presented the following arguments against this reasoning: When an individual is infected by vaccinia, he is for a time immune to small-pox, and on the other hand an individual infected by small-pox is subsequently for a time immune to infection by vaccinia. Are small-pox and vaccinia therefore identical diseases? We will all most likely admit that these two diseases are related but not identical. Again, Metchnikoff and Besredka have shown in their very pretty work on immunity in typhoid fever, that when a monkey is once infected by paratyphoid fever, such monkey is immune to typhoid fever; yet paratyphoid and typhoid are related but not identical diseases. Above all I insisted that it was not justifiable to speak with positiveness on the identity of two diseases, especially when the causative organism which most likely produced the diseases under discussion were absolutely unknown.

I should like to say a few words on the subject of the transmission of typhus fever, though it is hardly possible in the five minutes allowed me by the chairman to discuss any of the many interesting subjects suggested by the papers of the evening. Therefore I may only hint at this subject. Nicolle was the first to show that a monkey could be infected experimentally with typhus fever by means of the bites of body lice which had been permitted to feed on a typhus fever subject. Ever since then it has been accepted that typhus fever is transmitted by the agency of the body louse. Anderson and Goldberger not only confirmed this observation but showed that the same results occurred when head lice were used as the agents of transmission. It is very likely that the epidemic form of typhus fever is spread in this way. It would appear from our own observations that this means of transmission is not important in the mild or endemic form of the disease, and that there is most probably some other mode of transmission. These observations are as follows: As was shown by Nicolle, the body louse cannot live at higher temperatures than 12° to 14° C.; it thrives only in the cold when it is most active and presumably most virulent. Anderson and Goldberger showed that when lice were collected and kept in a test-tube in the pocket of the experimenter they died even at such a moderate degree of warmth. It is true that epidemic typhus occurs most frequently in the colder months, as anybody may see who consults the statistics of Murchison, who states that out of 18,230 cases of epidemic typhus fever observed in the London Fever Hospital between the years 1847 to 1870, only 3,730 occurred in the warm months of the year; that July and August furnished by far the least of these. In other words, only about one fifth of typhus fever cases appeared in the summer months. Contrary to the seasonal preference observed in the epidemic variety, the form of the disease which the authors have called the endemic form, shows that by far the largest number of cases occur just in the hottest months of the year, months in which the body louse is not at all active. All my papers show this feature; in fact in the last reported series at the International Congress, of 62 cases reported in 1911 and 1912, 42 occurred in the warm months; 28 of these appeared in the months of July, August and September. The warm months from June to November in our cases furnished twice as many cases as all the rest of the year. Again, if the body louse or even the head louse were the infecting agent in the mild form of the disease, how is it that no family infection appears, that the husband is most rarely infected by the wife, or the wife very rarely by the husband? In over 400 cases of this form of the disease, there has been only one case in which the husband and wife were infected, one by the other. This is a feature which it seems to me requires further study and investigation, for it seems likely that there is some other means of transmission than by the vermin just accused. Finally before I sit down I want to pay my tribute of congratulations to the authors of this evening's epoch-making work in our knowledge of typhus fever.

DR. MELTZER: I cannot add anything of value to the discussion. I am not a bacteriologist and feel that I am not entitled to offer a statement to the effect that the three communications offered to us prove conclusively that the organism discovered by Dr. Plotz is the real cause of typhus, endemic or epidemic. But since you called upon me, Mr. Chairman, to add something to the discussion, I gladly say this: It gave me great pleasure to listen to the way, the scientific manner and the thoroughness in which these investigations were carried out, and that I greatly appreciate the scholarly and lucid manner in which the results were presented to us. We may congratulate ourselves on the accession to our ranks of this group of young investigators.

DR. FRIEDMAN: I understand that this is not a clinical evening, but a discussion of the origin of typhus. Five years ago I took part in a discussion of Dr. Brill's paper and I must say that I was the first one to state definitely that the symptom complex he described was nothing else but typhus. Hardly anyone then wanted to agree with me. They said they had observed these cases for fourteen years and they were not typhus. Dr. Warren Coleman alone, in a personal communication, said he thought the disease might be a form of typhus. I do not minimize Dr. Brill's work. Men who should know more about typhus than Dr. Brill have made mistakes. In Manchuria, a St. Petersburg professor, Dr. Botkin, found a deviation in type and described it as a different disease. It was clear to me without any bacteriological evidence that Dr. Brill's cases were not different from typhus. In regard to the louse, a Russian physician inoculated himself with the blood of a typhus patient and eighteen days later developed the disease. This inoculation from man to man shows that typhus transmission can occur without the body louse. But I believe the body louse increases the virulence of the disease. This is seen in Russian prisons. Everyone may get typhus from one case, the prisons being filled with lice, and I believe the infection derived through lice is more severe.

DR. LOURIA: There is nothing for me to add. From the clinical viewpoint I have to recall cases of typhus fever which I observed in my student days. It is a common disease in Russia. I saw it constantly in my early student and hospital days. When my attention was called to the work of Dr. Brill I was impressed with the fact that these cases looked like what I had seen, but with differences; that is, they run a mild course. Although these two types have been proven bacteriologically and experimentally identical, yet I feel there must still be something missing in the work. I feel, however, that the answers will be presented in time by these able workers.

Dr. Mandlebaum: I should like to draw attention to the great importance of Dr. Brill's work on the isolation of cases of endemic typhus fever—so-called "Brill's disease"—from other fevers. Were it not for his work and the observations that followed, the identity of "Brill's disease" to typhus fever would not likely have been made, and the epidemic cases which were held at quarantine would surely not have been investigated in connection with this work.

When the cause of an acute infectious disease is discovered, one naturally looks for some serum or vaccine for the treatment, or, what is more important, for its prevention. We have heard an allusion made to a prophylactic vaccine. I think I may be permitted to state here that some members of the commission who have already gone to Serbia, and others who intend to go, have been injected at their own request with the vaccine made by us, without any guarantee, of course, as to its efficacy. The efficacy of any vaccine, as we all know, can only be determined when employed in an epidemic.

Dr. Libman: In regard to the question of secondary invaders,—the suggestion has been made that the bacillus described to-night may be an analagous finding to that made in yellow fever by Sanarelli. The latter studies were mostly made after death and in the main mixed cultures were obtained. The work in typhus fever was all done *intra vitam* and the organism was always obtained pure. The serological studies also prove the relationship of the bacillus to typhus fever. If this organism were not accepted it could be only as the basis that there were two etiological agents,—an assumption not warranted by experience.

The organisms when isolated may have lost their virulence because of their long stay in sugar media. If even without sugar they should be proven non-virulent we might make the assumption that the louse is of importance for maintenance and development of virulence.

As to Dr. Brill's remarks on the question of transmission,—either endemic typhus is not conveyed exclusively by lice or we do not yet understand all the features of louse transmission.

The discovery of Dr. Plotz did not depend on chance observation. I have been informed that he had the problem of Brill's disease in his mind when he was a medical student, and that he entered the laboratories of the Mount Sinai Hospital to work on the etiology of the disease. The organism was obtained the first time he tried good methods for it, which is unusual. When he showed me the organism I believed that he had found something of importance and advised him to go on. I consider it a privilege to have been associated with Drs. Plotz, Olitsky and Baehr in the work, although I consider myself more in the character of a watchman than anything else.

Dr. Baehr: Dr. Libman has answered most of the questions. In reply to Dr. Park, we wish to say that we have tested the susceptibility of other laboratory animals, such as rats, mice and rabbits and some observers have tested cats, dogs, sheep and goats. No other animals except monkeys and guinea pigs respond actively to the inoculation of a typhus virus. In reply to Dr. Brill as to the possibility of transmission of the endemic disease by other insects than the body louse,—we prefer not to commit ourselves at present. There may be another agent. Thus far it has only been possible to demonstrate transmission by means of the body louse, and the presence of the latter upon many of the endemic cases of typhus fever, even at the time of their reception in the hospital, is therefore very significant.

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FOREIGN BODIES IN GIANT CELLS[1]

F. C. WOOD, M.D.

(From St. Luke's Hospital, Pathological Department. F. C. Wood, Director)

The patient from whom the material to be shown was removed was a woman (C. B., St. Luke's Path. No. 11022), twenty-six years of age, who about a year previous had had a paraffin injection in the neck for cosmetic purposes. Owing to the considerable hyperplasia which resulted from the injection, it was necessary to remove the mass surgically.

The tissues showed the usual morphological appearance found after such injections, consisting in a very considerable inflammatory reaction of a chronic nature with many lymphocytes and plasma cells lying in a meshwork of granulation tissue. Scattered throughout this granulation tissue were numerous giant cells, some surrounding globules of the paraffin, and others not showing any included structure, while a considerable proportion contained stellate bodies which occupied the center of the cells. These bodies consisted of a central mass, 2 to 3 μ in diameter, from which sprang curved spicules, fifteen to twenty in number. The diameter of the whole body was 20 to 25 μ. These bodies stained fairly well with hematoxylin and somewhat more deeply with Weigert's elastic tissue stain. In the writer's opinion, they probably represent condensed protoplasmic shells about crystals of paraffin, or possibly fatty acids. Mallory and others consider that they are fibrinoid in nature.

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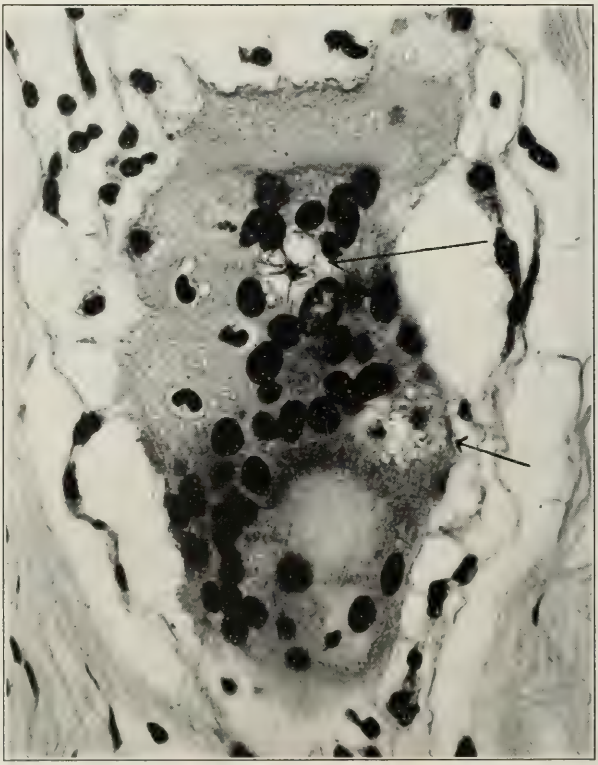
[1] Read at the March meeting of the Society.

The literature concerning these structures, which have, of course, only an academic interest, is not very extensive. The first report was that of Ris, who described similar giant cells in an omental cyst removed from the peritoneal cavity of a man, fifty-one years old. The cyst was about 7 cm. in diameter, with a wall 4 mm. in thickness. The lining of the cyst was composed of granulation tissue with many cholesterin crystal giant cells and also giant cells containing the peculiar stellate structures under discussion. Ribbert, who saw the preparations, thought that they were imperfectly developed portions of moulds (*Schimmelpilze*). These stellate bodies did not stain with the ordinary nuclear stains nor with Weigert's fibrin stain. They measured about 15 to 25 μ in diameter and were composed of curved needles radiating from a central homogeneous area. About one-third of the giant cells contained these bodies.

The next report was that of Wolbach, who stated that in 900 autopsies he had found five cases in which giant cells contained small radiating inclusions. In the first case the structures were present in the lungs; in the second, in the lymph nodes; in the third, in the lungs and lymph nodes; in the fourth, in the spleen, liver, and lymph nodes; and in the fifth, in the lung, spleen, and liver. In one case they were found in the peripheral sinuses of the lymph nodes. The smaller forms were often included in endothelial leucocytes and occasionally within endothelial cells which were attached to the walls of the lymph spaces. Wolbach called attention to the fact that these inclusions were not parasites though they resembled structures occasionally encountered in experimental lesions produced by certain fungi. He held that it was impossible that they should be artefacts. For the material which he studied the best stain was found to be Mallory's phosphotungstic acid hematein; Gram's stain colored the central body and the spines a deep blue, the intervening material taking the counterstain; and with Mallory's eosin-methylene blue the spines and central body were stained a deep purple and the intervening portion a pink color. The bodies were not soluble in caustic potash or in hydrochloric acid, and were not stained by silver nitrate in frozen sections of formalin material. Scharlach R and osmic acid did not stain them nor did iron hematoxylin or Weigert's elastic tissue stain. The iron reaction was negative.

Vogel described similar bodies in giant cells in a case of obliterative capillary bronchitis in a child eleven years old. The giant cells were found in the connective tissue replacing the bronchi and the perivascular connective tissue. They contained one to three star-like bodies which suggested crystals; took a rather faint stain with hematoxylin, and colored best with Weigert's stain for elastic fibers. Fat, fatty acids, and iron could not be demonstrated in the giant cells. Vogel thought that these bodies were probably not altered elastic tissue, but he could give no suggestion as to their nature. He referred to articles by Ssudakewitsch and Rona, but the structures figured by these two authors do not in the least resemble the stellate bodies under discussion.

Iwanzoff found similar structures in a myomatous uterus removed from a woman, fifty years old. In this myoma, which showed a considerable amount of hyaline degeneration, there were areas of lymphoid infiltration containing irregular giant cells. In some cases these giant cells were so abundant that they formed a large part of the nodule. There was nothing peculiar about the giant cells, except that when stained with Weigert's elastic stain they showed peculiar radiating structures previously described by Wolbach. They were not bacterial in nature and did not stain except with Weigert's stain. The writer discusses the question of whether these are phagocyted elastic tissue fibers, such as are frequently seen in tuberculosis of the skin, whether they are parasitic inclusions, or whether they are astrospheres. He referred to a paper by Wakabayashi in which the astrospheres in tuberculous and other giant cells are described, and thought that these bodies closely resembled the structures which he had reported.

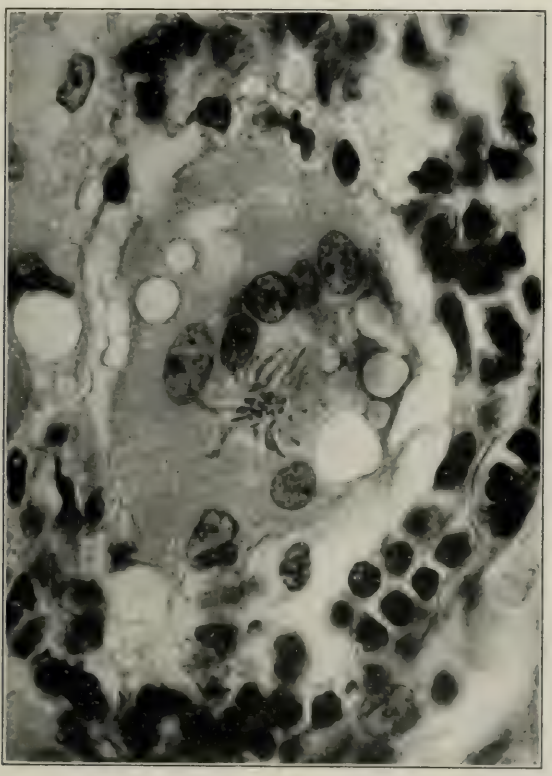


Stellate Bodies in Giant Cells. × 750.

Mallory in his text-book shows a giant cell (Fig. 120, page 207) from a case of leprosy, which contains a spiculated body of the type under discussion. He gives no details except the legend under the cut. In another cut he shows similar spiculated bodies

(Fig. 465, page 613), the text referring to these reading as follows:

"Spiculated bodies have been found in the spleen in a few instances inclosed in endothelial leukocytes and giant-cells. They may be quite numerous and occur in foci or diffusely scattered. The lesion resembles miliary tuberculosis, but no necrosis is produced. The bodies seem chemically to be of a fibrinoid character. They are evidently not the cause of the lesion, which is probably of infectious origin, but a secondary formation."



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Discussion:

Dr. Larkin: What relation do these bodies bear to the inclusion bodies in cancer?

Dr. Wood: They differ from degenerating cells in carcinoma that I have seen, and I suspect that they may be fatty acid crystals about which condensation of protoplasm has taken place. If they were cellular fragments they would, I think, be more often met with; on the contrary, these stellate bodies are very rare.

CONGENITAL SARCOMA OF VAGINA

A. B. EISENBREY, M.D.

(From St. Luke's Hospital, Pathological Department, F. C. Wood, Director)

In 1911, reports of one hundred and two cases of sarcoma of the vagina were collected by MacFarland of Philadelphia. Of these cases, thirty-four were of the type known as sarcoma botryoides, or grape-like sarcoma. All but one of these cases proved rapidly fatal. They occurred in young children from three months to five years of age. In one case the tumor was noticed at birth. It was shown to be essentially a disease of childhood. On account of the diversity of cell types found it was regarded by many as a congenital tumor.

The case I wish to report was three and one half years of age, a patient in St. Luke's Hospital, on the service of Dr. Abbe. One year previously the family noticed that on crying or making efforts at stools a polypoid mass projected from the vagina. An operation was then performed and the child remained well for about a month, when there was a recurrence. Four operations were performed before her admission to St. Luke's Hospital in March, 1915, at which time the vaginal growth was again removed. While in many cases reported, the tumors were found to be single, the growth in this case was made up of multiple tumors, and involved the entire surface of the vaginal wall. There was apparently some growth also on the margin of the cervix. The child had marked difficulty in micturition and defecation. Palpation through the rectum revealed a sausage-like mass which filled and appeared to be confined to the vagina.

The specimens received for examination were six or eight rounded, pedunculated masses, resembling nasal polyps, pinkish in color, and quite oedematous. In some the pedicle was fibrous. All of the growths were covered by epithelium, which showed a few erosions. Two or three of the small masses consisted of vaginal mucosa containing areas of minute soft nodules beneath the epithelium. Microscopic sections showed a typical polyp-like structure, some of the denser areas consisting of closely packed spindle cells. A few areas of distinctly myxomatous type were found. The predominating cell was of the spindle type. Many young striated muscle fibers were present in some of the polypoid growths, quite apart from the blood vessel walls and the vaginal musculature. They occurred singly and in small bundles. It is on this finding that the "congenital" nature of the growth is based.

Discussion:

Dr. Lambert: I am not at all familiar with the first type of sarcoma of the vagina. On the basis of the microscopic appearance I should hesitate to call it sarcoma. I should rather call it a benign "mixed" tumor or teratoma.

Dr. Eisenbrey: There were a great variety of cells described in connection with this type of tumor. The name was given on account of the grape-like nature of the growths. In some, the clusters seem definitely cystic. Some were myxomatous, but the predominating type of cell was the spindle variety; some showed round cells; some were combinations of the two; three cases were fibromatous in type, others fibromyxomatous; all of these types, however, have been included under sarcoma botryoides. In a large number there were some muscle fibres and in some cartilage. MacFarland's own case did not contain muscle fibers; it was made up entirely of spindle cells, and areas of œdema and degeneration were numerous. They are certainly malignant growths.

Dr. Levin: This tumor seems to be similar to the mixed tumors of the testicle where we find various kinds of cells. They are probably fœtal in origin.

Dr. Eisenbrey: That is the reason I classified this case as a "congenital" tumor.

XANTHOMATA OF TONGUE AND ELBOWS

A. B. EISENBREY, M.D.

(*From St. Luke's Hospital, Pathological Department, F. C. Wood, Director*)

Xanthoma of Tongue

The specimen was from a man thirty-five years of age, a heavy smoker, a patient in St. Luke's Hospital, on the service of Dr. Abbe, in October, 1914. Two months previously he noticed a hard, painless nodule about 0.6 cm. in diameter on the dorsal surface of the middle of the tongue, slightly to the right side. On section the growth showed distinct demarcation from the surrounding tissue. It was covered by epithelium, and was of firm consistency. Many cells could be scraped from the cut surface. It was whitish in color, and no yellow pigmentation was noticed. Microscopic sections showed groups of characteristic fat-containing cells lying in meshes of a rather dense connective tissue stroma. Under the polarizing microscope the fat proved to be of the double refractile variety.

Xanthoma of Elbows

These specimens were from a young Russian woman, twenty-six years of age, a patient in St. Luke's Hospital, on the service of Dr. Downes. Since childhood she had had yellowish lumps on her elbows. Two months before admission in February, 1915, she noticed the lumps on her elbows were becoming larger, and that some small ones were beginning to appear on both knees. There was no history of previous illness, but the patient was quite neurotic in type, and had physical signs of incipient Graves' disease. Tumors removed from elbows at operation showed well encapsulated, bright yellow, fibrous nodules, which were not attached to the skin. The loose tissue about the nodules and the skin also showed a bright yellow color. Microscopic sections showed typical fat-containing cells lying in meshes of a rather dense fibrous stroma, and also a few fatty cells and fat masses that were loose in the tissue about the nodules. The fat was doubly refractile with the polarizing microscope. Many crystal spaces with giant cells about them were present in some of the dense portions of the nodules.

Discussion:

Dr. Pappenheimer: Are these spaces cholesterin crystals?

Dr. Eisenbrey: Yes. They were considered to be cholesterin and in the fresh state sheaves of fatty acid crystals also were found.

Dr. Lambert: Isn't the tongue an unusual place for xanthoma?

Dr. Eisenbrey: Yes. It is generally found in the abdominal organs, the trachea and pericardium. The small xanthomata about the eyes that occur in old people are probably the result of degenerative processes and not neoplasms. The diagnosis in these cases was made on the nature of the fatty cells and the double refractile nature of the fat to polarized light.

Dr. Lambert: In the second case, I should think there might be a question as to its being xanthoma; occasionally cells containing chlosterin esters are found in other growths. Was the cholesterin content of the blood determined in these cases? Contradictory results regarding this question have been reported. In a recent paper Rosenbloom (Arch. Int. Med., 1913, XII, 395) reported normal cholesterin content of the blood in a case presenting multiple xanthomata.

EOSINOPHILIC INFILTRATION OF THE PANCREAS

HENRY A. RILEY, M.D.

(From the Department of Pathology of the College of Physicians and Surgeons, New York City)

The case which is the subject of this report possesses interest in that as far as can be ascertained, it is the only case on record which presents an eosinophilia limited to the pancreas alone and existing in that organ without discoverable cause and independent of a generalized eosinophilia.

Before describing the histological characteristics of the organ involved, it may be well to consider briefly the cause of generalized and local eosinophilia. The main authority consulted is the exhaustive study on general and localized eosinophilia by E. Schwartz in the *Ergebnisse der allgemeine Pathologie*, 1914, Vol. 17, Part I.

General Eosinophilia: Drawing his facts from many sources, Schwartz places the normal limits of the eosinophilic cells in the circulating blood between 2 per cent. and 4 per cent. in adults, 2 per cent. and 5 per cent. in children. Race, sex, age, temperature of the environment, pregnancy and parturition seem to bear no direct influence upon the eosinophile cells. The condition of nourishment appears to exert some influence, starvation reducing the number of cells, which, however, rapidly returns to normal upon the restitution of sufficient nourishment.

Splenectomy or ligation of the splenic vessels is followed by an immediate decrease in the number of cells, then a rise to a supernormal figure and then a slow drop to a subnormal figure which is then maintained.

The effects of many and varied toxic agents have been studied. Those which produce an increase are antimony, CO, CO_2 and pilocarpine. Hæmotoxic substances seem to exert no constant effect upon the number of eosinophiles. Anaphylactic shock is regularly followed by a marked and protracted eosinophilia. This phenomenon is considered to be the explanation of the eosinophilia observed in asthma, hay-fever, many of the dermatoses and certain obscure intestinal phenomena which have been held by many to be due to localized anaphylactic conditions.

Disturbances of the glands of internal secretion do not seem to exert any constant effect upon the eosinophiles except in the thyroid; hypothyroidism being fairly constantly accompanied by an increase, hyperthyroidism by a decrease in the number of eosinophiles. Certain nervous diseases are accompanied by an eosinophilia, among which may be named epilepsy, especially in periods immediately following active seizures, dementia præcox, paranoia, and many psychoses of various origins.

The conditions which produce the most striking examples of eosinophilia are those associated with parasites. The site of the infection does not seem to influence the blood picture, for similar extreme examples are found in all of the groups of parasites whether they exist in the intestinal canal, the parenchymatous organs, the urinary system, the muscles, or the blood. They differ markedly in their eosinophilic properties, however, some of the parasites being unable to stir up any response, while others produce such a profound effect that the eosinophilic cell may occupy an almost exclusive position in the composition of the white elements of the blood. In general, higher values are observed in children than in adults. Those parasites which are

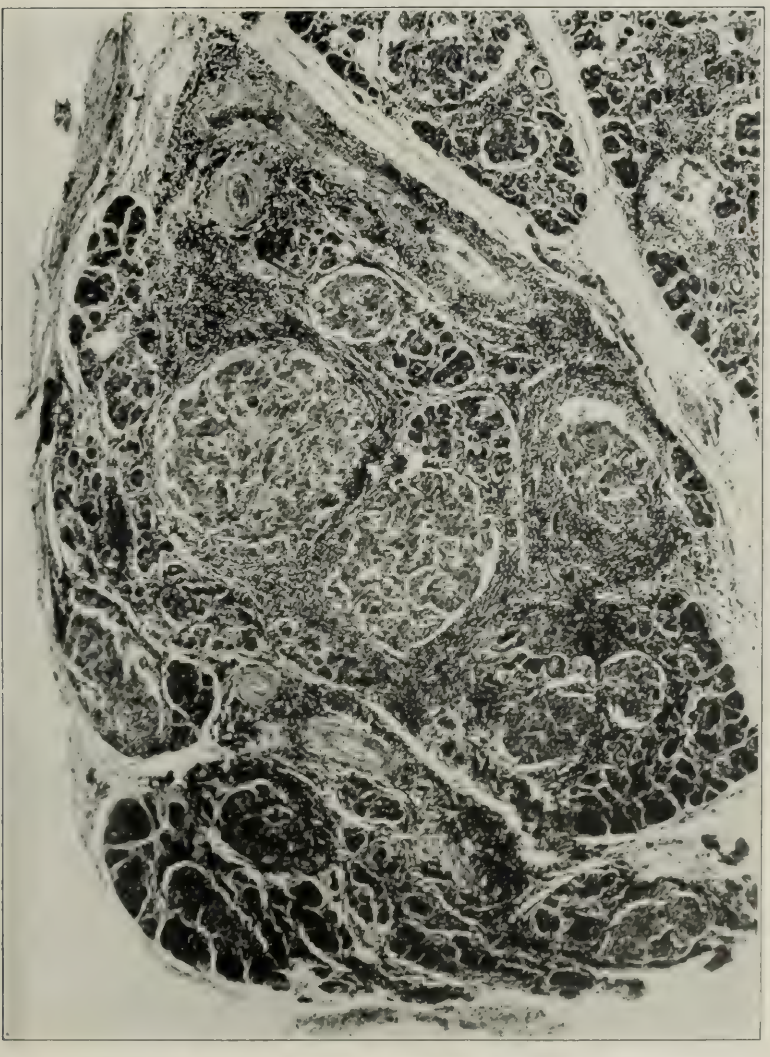

FIG. 1. Low power picture showing islands of large size and eosinophilic infiltration of the surrounding tissues.

capable of passing into encysted form gradually show a diminishing influence on the eosinophiles. If, however, the contents of the cyst gain access to the blood stream either by rupture, or by aspiration and injection, an immediate eosinophilic response results. Of the intestinal parasites, the *Ankylostoma duodenale* stands at the head in its ability to produce an eosinophilic response, values as high as 80 per cent. having been recorded. When it is considered that at the same time there is a marked rise in the total number of leucocytes, it can readily be seen that enormous numbers of new cells have been added to the circulating blood. Malaria in its chronic forms has been known to produce 20 per cent. of eosinophiles.

Certain of the infectious diseases present instances of moderate increases in the number of eosinophiles,—rheumatic fever, scarlet fever, vaccinia, the post-critical period in pneumonia when the eosinophiles which have heretofore been completely absent from the blood reappear. In gonorrhea, especially with the incidence of complications, a moderate increase in the number of eosinophiles has been observed, while extreme values of 25 per cent. have been recorded. Amœbic dysentery has also shown high figures, 47 per cent. having been reported in one case.

In tuberculosis we find the eosinophiles playing a rôle of some prognostic importance for they disappear from the blood in cases of advancing involvement by the tubercle bacillus and reappear only when the reparative processes of the body have succeeded in outstripping the ravages of the disease.

The list of dermatoses with which eosinophilia has been associated is a long and formidable one. It is an almost constant accompaniment of urticaria, eczema, prurigo, iodide and mercury erythemata, and all vesicular and bullous diseases. Psoriasis shows an eosinophilia only inconstantly, but it is invariably present in the dermatitis exfoliativa which often follows psoriasis.

A general eosinophilia is also seen accompanying many of the granulomata and neoplasms. Prominent among these is Hodgkin's disease where figures up to 28 per cent. may be encountered. Curiously enough, higher values may be met with after *x*-ray treatment, percentages up to 68 per cent. having been reported. General sarcomatosis and carcinosis may produce a generalized eosinophilic response even to 45 per cent. in cases of the latter. Lymphosarcoma may also produce a general blood-reaction, but to a less degree.

Local Eosinophilia: The eosinophiles are found widely scattered throughout the body under physiological conditions, both in the fluids of the body and also in the tissues. In the hæmolymph glands they are more numerous than in the ordinary lymph glands. They are said to be absent from the mucous membrane of the œsophagus and stomach but are present in considerable numbers in that of the intestine. A diet mainly of meat or one containing many irritating components is said to increase the number of the eosinophiles in the mucosa. During the digestive periods the number in the spleen increases, while in fasting periods only a few can be found. When present they are found only in the pulp. There are no recorded investigations as to their presence in the salivary glands or the pancreas.

They exist in only moderate numbers in the walls of the respiratory

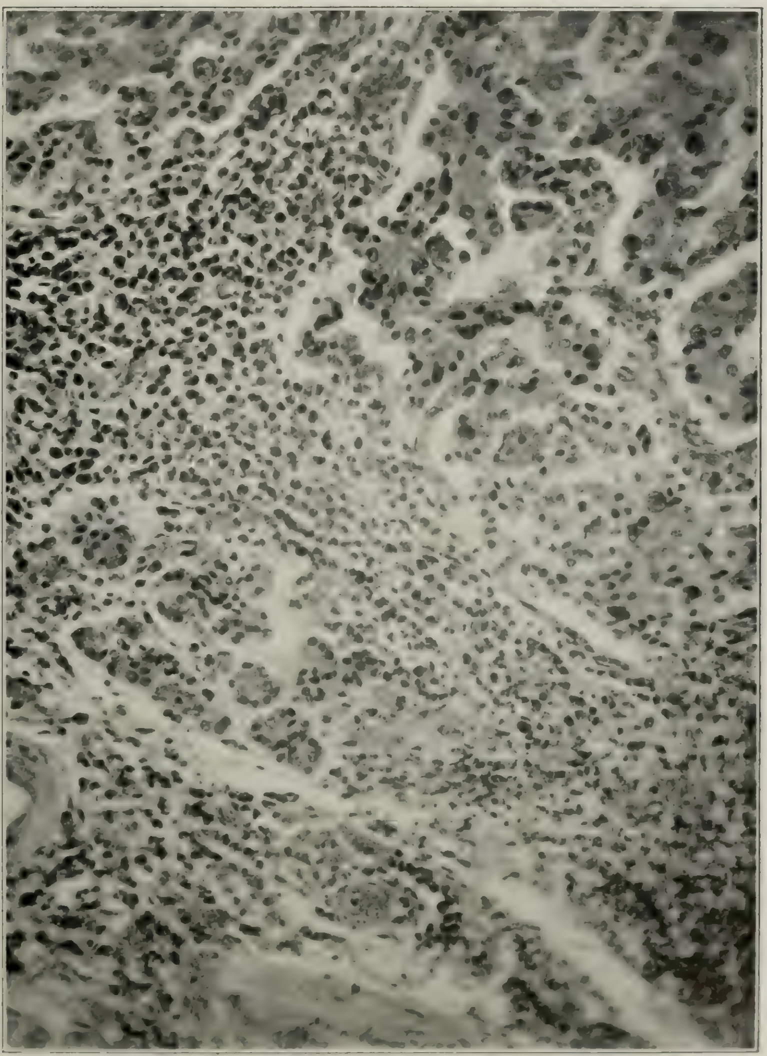

FIG. 2. High power of same showing disordered structure of the islands and the infiltration of the surrounding tissues by eosinophiles.

tract, but are said to present accumulations about the mucous glands of the bronchi.

They may be found in varying numbers in the thymus increasing up to the seventh month and then receding.

In lesions of the gastro-intestinal tract they may be materially increased in number, being found in catarrhal gastritis, atrophic gastritis and especially in tissues showing neoplastic changes. They are supposed to bear a direct relation to the increased mucous secretion of the disordered mucosa rather than to the processes of disintegration. In some forms of appendiceal inflammation they are quite numerous. These accumulations in the mucosa of the intestinal tract are attributed to local immunity reactions comparable to the generalized eosinophilia associated with anaphylactic phenomena.

They are present in the myocardium of cases dying during the course of diphtheria. They are not found in cases proving fatal prior to the seventh day of the disease. Attempts to explain them on the basis of anaphylaxis resulting from the injection of antidiphtheric sera have not been successful, for they have been found as frequently in those who have been and those who have not been treated with serum. They are not present in increasing numbers in comparison with the number of serum injections, and they bear no relationship to the clinical severity of the case. They are found closely gathered about the smaller blood vessels but not in the region of blood extravasations nor near the foci of degeneration.

They are inconstantly present in the pus arising from non-specific inflammations, but in the pus from gonorrheal processes in any site they are abundantly present, increasing in direct relation to the duration of the process until they may make up almost the entire bulk of the suppurative material. In the nasal and bronchial secretions from asthma and hay-fever they are very numerous and may in these instances be largely mononuclear in character. In asthma it has been noted that large numbers of the leucocytes found in the urine may be eosinophiles. The fæces in cases of intestinal helminthiasis may contain large numbers of eosinophilic leucocytes. Pleural exudates often show increasing numbers of eosinophiles as the inflammatory process recedes.

The parasitic lesions of the skin show a local eosinophilia, the cells being arranged either in groups or scattered diffusely through the tissues. Often they are gathered about the smaller blood vessels. In cases of infection with *Trichinella* the eosinophiles are not strictly limited to the immediate vicinity of the parasite, but they may be found throughout the interstitial inflammatory tissue. In lymph node involvement by *Filaria* the eosinophiles may be found in large numbers occupying both the lymph sinuses and the lymph cords. The lymphatics may also be found to contain great numbers of eosinophiles. *Balantidium coli* is also capable of producing a marked eosinophilia, the infected areas of the colon containing many of these cells.

The dermatoses in a similar manner show a local eosinophilia, the tissues surrounding and underlying the lesions often being tightly packed with eosinophiles. The skin diseases capable of producing a local eosinophilia are identical with those which are responsible for the similar generalized blood reaction.

The local eosinophilia about neoplasms is often of considerable importance. This question presents many obscure and puzzling features for no one constant factor can be found exercising any marked influence upon this specialized tissue reaction. One form of tumor may in one site be accompanied by a marked eosinophilia only to find a similar reaction completely lacking in a similar tumor in another region, and one form of tumor in a given region may supply many eosinophiles, whereas a different tumor in the same tissue will show none. In general, however, it may be stated that those tissues which are exposed to the greatest irritation are those in which the eosinophiles are the most numerous.

The carcinomata are fairly regularly accompanied by a moderate eosinophilic infiltration, the uterus being the tissue which is capable of furnishing the greatest numbers of these cells. They are also present to a lesser degree in sarcomata of all types. They are usually infrequent in the hypernephromata. Benign growths may also show an eosinophilia, but less regularly and to a slighter degree, than the malignant growths.

The lesions of periarteritis nodosa show regularly a diffuse admixture of eosinophilic cells.

CASE REPORT

The child whose pancreas is the subject of this paper was born on the 25th of March of this year at the Nursery and Child's Hospital. It was the last of fourteen pregnancies, one of which terminated in a still-birth; all the others however, including this one were normal in all respects. The child was living until labor began, but as it was a very large child, weighing over ten pounds and being a breech presentation with a long difficult labor, extraction was protracted and the child was born dead. There were no external abnormalities, no stigmata of syphilis, and all the organs were found in an approximately normal condition. The pancreas was grossly quite normal. The cause of death was ascertained to be a fracture between the third and fourth cervical vertebrae, consequent upon forcible extraction in the breech position. No suspicion of anything pathological was aroused in the routine microscopical examination except for a slight degree of degeneration in the parenchymal cells of the liver, kidneys and adrenals, until the pancreas was reached, where a most unusual and interesting condition was found. This change affected all the constituents of the pancreas, the islands of Langerhans, the interstitial tissue and last and least the acinar tissue. The same condition existed in all parts of the organ examined.

The islands were enormously increased in size and almost visible to the naked eye. They were much distorted and their usual orderly internal structure was much obscured. They were made up chiefly of two types of cells: (1) A cell which comprised the main bulk of the island and was large with a granular deep staining cytoplasm and an ill-defined cell outline. The nuclei differed much in size, some were very large indeed and presented a clear protoplasm, a definite chromatin network, and in some cases a distinct nucleolus. The cytoplasm of the cells in many areas resembled a syncytium. These cells suggested in many of their properties neoplastic cells. (2) The second type of cell was much less numerous and was arranged principally about the periphery of the islands. It had a small darkly staining nucleus, more or less oval in outline, in which no chromatin network could be made out. Its cell outline was somewhat more definite than that of the preceding cell, and its cytoplasm took a pinker stain. Occasional eosinophiles could be seen in among the cells of the island. In the spaces which surrounded the capillary loops could be seen numbers of beautifully granulated eosinophiles, which were in greater part multilobular, although mononuclear cells were present in fair numbers. In the connective tissue of the capillary loops the eosinophiles seemed to be surrounded by delicate connective tissue strands. The connective tissue of the pancreas presented the most extraordinary eosinophilic infiltration, the entire tissue having been simply converted into eosinophilic tissue, each cell, in many instances, surrounded by delicate connective tissue fibrils. Between the eosinophile cells could be seen occasional connective tissue cells and only very infrequently a polynuclear leucocyte. The tissue was so densely infiltrated with eosinophiles that it was difficult to demonstrate any particular condensation about the blood vessels. The capillaries were engorged and the lining endothelium seemed enlarged and swollen, but only occasional eosinophiles could be found within the capillaries. There was a large amount of free eosin-staining granular material present in the section. Scattered here and there singly and in small clumps between the cells could be seen long needle-like crystals pointed at each end and somewhat thicker at the middle, which took a pink stain, and seemed to be typical Charcot-Leyden crystals. The acinar tissue was much reduced in amount and the acini were in large part isolated from one another by a uniform increase in the amount of inter-acinar connective tissue which appeared oedematous and was loaded with eosinophiles. The majority of the acinar cells appeared quite normal but occasional vacuolated cells and indeed entire degenerating acini could be found. The eosinophile cells did not invade the acini.

Attempts to clear up the etiology of this condition have been without success. Tissue stained by the Levaditi method and examined for spirochaetae has not disclosed any of these organisms. Efforts to find bacteria in preparations stained by the Gram method and with Giemsa's stain have also been unsuccessful.

The question of the origin of the eosinophiles, whether by emigration from the blood vessels or by local production will be left open.

It is equally impossible to give any definite opinion as to the underlying cause for this peculiar picture, but it would seem that it was associated with the islands of Langerhans. The appearance of the cells of the islands indicated that some unusual process was in course there and it is conceivable that some abnormality in their metabolism may be responsible for the eosinophilic infiltration.

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Discussion:

DR. PAPPENHEIMER: I would like to ask the Society for suggestions about this case. It seems to be an unusual condition and none of the long list of possible causes of eosinophilia, which Dr. Riley has cited seems applicable here. In no inflammatory condition with which I am familiar, is the exudate composed entirely of eosinophilic cells. There are, of course, a great many eosinophiles in gonococcus infections of the mucous membranes, but in Dr. Riley's case the exudate is exclusively eosinophilic. Active blood formation in and about the pancreas is not uncommon in new-born infants. In this case, however, the absence of other blood elements and of active hematopoiesis in the liver—and the fact that the eosinophiles are almost all polymorphonuclear makes it certain that the areas are not to be regarded as blood-forming islands.

DR. EISENBREY: The only thing I have seen resembling this was in a case of Hodgkin's disease, where the eosinophilic infiltration was very nearly as marked. Occasionally in epithelioma of the cervix you see the exudate composed almost entirely of eosinophiles, but these conditions have no bearing on this particular case.

PATHOLOGICAL FINDINGS IN A CASE OF AMAUROTIC FAMILY IDIOCY

SARA WELT-KAKELS, M.D.

As it is the writer's intention to publish later a paper upon this subject *in extenso*, only a brief abstract of the report will be given on this occasion in connection with the demonstration of sections from a case of amaurotic family idiocy.

A general review of the contributions to the present knowledge of the subject will be omitted at this time and attention will be given to the discussion of the case. Pathological findings were demonstrated by microscopical sections of the brain, spinal cord, and spinal ganglia of a girl who died from this disease when 23 months old. The nervous tissues showed widespread degenerative processes involving the gray nerve cells, consisting of balloon-like swellings of the cell body and dendrites with the disappearance of the Nissl bodies and a peripheral displacement of the nucleus. In more advanced degeneration, the cells appeared like structureless masses. These alterations of the cells were especially well demonstrated in sections stained with Bielschowsky's stain.

Sections of the ductless glands of the patient were also exhibited. The thymus showed marked increase in connective tissue, mainly in the septa. Hassal's bodies appeared to be increased in amount, with disappearance of thymic parenchyma.

The thyroid sections showed no changes.

The adrenals showed slight parenchymatous degeneration; the cortical substance was somewhat shrunken.

The hypophysis showed increase in the number of acidophilic cells.

The examination of the ductless glands seemed to be very opportune, since Dr. Harlow Brooks reported marked alterations in the thymus, adrenals and pituitary glands in a case of amaurotic family idiocy, juvenile type. These lesions suggested to Dr. Brooks atypical functional activity and a possible causal relationship in this disease. The findings in the ductless glands of my case did not seem to justify the assumption that they were either direct or indirect factors in the causations of this fatal malady, nor were the lesions found in the endocrinous glands pathognomic for amaurotic family idiocy, infantile type.

A careful comparative study of ductless glands from children of approximately the same age, which, by courtesy of Dr. A. M Pappenheimer I was given the opportunity to examine at the Nursery and Child's Hospital, seemed to show that similar changes in the endocrinous glands occurred in children who succumbed to other diseases.

Discussion:

DR. PAPPENHEIMER: Was the sympathetic system investigated?

DR. WELT-KAKELS: No, so far as I know, in none of the cases.

DR. LEVIN: Did I understand that some chemical examination had been made of the central nervous system, or not? Was any thing found chemically that would indicate where the trouble lay? Most probably this is a metabolic condition, and not an infection. In this case some abnormal products in the brain may indicate the condition.

DR. WELT-KAKELS: Mott found diminution of sulphur and phosphorus. This was not corroborated by Heiman, Cohn and Bookman.

MORPHOLOGICAL CHANGES INDUCED IN TUMORS UNDER THE INFLUENCE OF ROENTGEN RAYS AND RADIUM

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The physical properties of Roentgen and gamma rays emitted by radio-active substances are completely analogous to the rays of light. Both kinds of rays represent, in accordance with the recent physical conceptions, waves of ether. The wave length of actinic rays, i. e., of Roentgen and gamma rays, are a thousand and more times shorter than the shortest ultra-violet rays of light. As a result the rays of light are absorbed completely by the first superficial layers of a substance which they traverse. The actinic rays, on the other hand, penetrate into deeper layers of the substance, and only a fraction of them is lost through absorption by the more superficial layers.

The biological action of the two kinds of rays is also analogous. This analogy is very evident in the so-called x-ray burn of the skin. When a large dose of soft x-rays is applied to the unprotected skin there takes place an erythema, blistering and ultimately the condition is qualitatively identical with a sunburn and only differs from it in degree. The browning of the skin which takes place as a result of an x-ray burn, or occasionally after x-raying of the skin even without the formation of a burn is identical with the pigmentation of the skin after a sunburn. Here again the difference is only in degree.

Fr. Bernig in a recent review states that the rays of light, or rather of the ultra-violet light, which is the only biologically active part of the spectrum, produce the following effect on the skin: Direct destruction of the cells; thrombosis of the blood vessels (through the direct influence of the rays on the endothelium and musculature of the vessels) and sero-haemorrhagic inflammation. The latter ends with the formation of hypertrophic connective tissue. It will be seen later that these morphological changes are very similar to the changes induced in tissues by actinic rays.

The analysis of a biological action of any agent is much simpler in the uncomplicated cellular structure of a plant or lower animal than in a vertebrate. A. Richards came to the following conclusions from his studies on the effect of x-rays on the rate of cell division in the early cleavage of *Planorbis*. The first effect of exposure to x-rays upon the rate of cleavage of the eggs of *Planorbis* is to stimulate mitotic activity. Following the phase of acceleration a phase of depression sets in; the end result is a marked retardation in the development of the egg.

In a vertebrate the actinic rays produce a biological effect on all the tissues of the organism, but the degree of injury varies greatly with the different tissues. It may be stated as a general rule that tissues consisting of less differentiated, younger cells also cells in a state of active proliferation are most deeply influenced by the rays. It is probable that in these complex vertebrate tissues the first effect of the rays also consists in an acceleration of the cellular functions. But it is very difficult to demonstrate such a phenomenon morphologically. Histologically one sees finally complete necrosis of the cell. Every tumor consists of undifferentiated young cells in a state of active proliferation, consequently a strong selective biological influence of the rays on tumors must be considered certain *a priori*. The morphological changes which take place in tumors under the influence of the rays differ with the different types of tumor and it may be well to consider them separately.

Carcinoma.—The first morphological changes which occur in carcinomatous tissue under the influence of actinic rays are observed in the tumor cells themselves and are manifested by the vacuolization of the protoplasm, pycnosis of the nuclei, karyolysis and complete necrosis of the cell. All this is accompanied by a round cell infiltration which replaces the destroyed carcinomatous cells. Somewhat later there begins formation of dense sclerotic connective tissue poor in blood vessels. This connective tissue formation may become very extensive, surround islands of carcinomatous cells and assist in the destruction of the latter. The following case of carcinoma of the sigmoid with metastatic dissemination in the peritoneum which came to autopsy after six months of continuous *x*-ray treatment demonstrates the importance and extent of this connective tissue formation.

The patient died of acute intestinal obstruction. At the autopsy there were found in the peritoneal cavity several loops of the intestines adherent by old adhesions to the posterior surface of tumor mass in the sigmoid. The peritoneum, especially in the pelvis, was studded with numerous white plaques, varying in size from one mm. to one-half cm. in diameter—the larger ones were quite firm to the touch. On section the mesenteric lymph nodes were rather small and reddish in color.

Microscopical examination of a section taken through two loops of the small intestine which were firmly bound together by old adhesions, showed that the adhesions consisted of a thick layer of connective tissue containing occasional nests of tumor cells. These nests were rather more abundant, however, in the muscular layers of the intestine than in the fibrous tissue connecting the loops. The tumor cells stained very deeply.

Another section taken from the edge of the colostomy wound showed a bit of skin with a wide zone of connective tissue beneath in which there were occasional groups of tumor cells in the muscular layers and mucous membrane of the intestine. There were relatively few groups of tumor cells in the muscular layers, but the mucous membrane was rather densely infiltrated with tumor. In the muscle, especially, one could see groups of tumor cells, apparently degenerated, in the midst of a mucoid material of the character that is found in the so-called colloid carcinomata.

The peritoneal nodules were composed of dense connective tissue, with occasional groups of tumor cells, which, as elsewhere, stained poorly. No follicles of colloid material were present. In some nodules no tumor cells were found.

Sarcoma.—The morphological changes induced in sarcomatous tissue by actinic rays is identical with those induced in carcinoma. The following case of a giant cell sarcoma of the lower jaw illustrates this effect. A small piece was removed for microscopic examination which showed a sarcoma with numerous giant cells and actively growing spindle cells. Following eight weeks of combined radium and *x*-ray treatment another section was taken from the same region from which the previous one was obtained. The section showed a very loose connective tissue, relatively poor in cells and beneath this a denser zone of cellular connective tissue. No giant cells or distinct spindle cells were present. A radiogram taken at the same time showed that the whole tumor was surrounded by a great deal of newly formed bone. Examination through the mouth showed the tumor to have completely disappeared and there was present instead a large cavity lined by a shell of bone.

Rhinoscleroma.—Infectious granulomata, whether tuberculous, syphilitic or of any other kind, are influenced by the actinic rays as lympho-sarcomata. The lymphoid cells are destroyed and replaced by dense sclerotic fibrous connective tissue. Rhinoscleroma is an infectious granuloma, characterized by the presence in the granulation tissue of the so-called Mikulicz cells. The latter are degenerated lymphoid cells, enlarged in size, inside of which may be found frequently the Frisch bacillus, the etiological factor. Under the influence of radium the observed cases showed a disappearance of the obstructing swelling of the naso-pharynx. The microscopic examination showed that the granulation tissue was completely replaced by dense connective tissue.

CONCLUSION

Thus the most generally observed morphological change in tumor tissue under the influence of radiation is the extensive formation of sclerotic connective tissue. Some observers maintain that this new connective tissue formation is the only direct effect of radiation. The destruction of the tumor cells is secondary and is due to lack of nutrition. This opinion cannot be accepted as true. In the first place, as was shown above, the first change noted in carcinoma was the destruction of the cells and only subsequently did the connective tissue form. Moreover, in certain conditions, for instance rodent ulcer of the skin, the epithelioma heals, is covered with skin epithelium and there is no formation of connective tissue.

Other investigators assert that the destruction of the tumor cells is the only direct affect of radiation. The formation of connective tissue, in accordance with this view is secondary to the accumulation of dead tumor cells and is analogous to formation of connective tissue about foreign bodies. This assumption is also hardly tenable. In the first place the amount of connective tissue formation in the peritoneal nodules of the carcinoma of the sigmoid reported above was entirely out of proportion to the number of carcinomatous cells present. Moreover, if such young connective tissue were formed only by the stimulus of dead tumor cells, then the actinic rays subsequently would dissolve this connective tissue as easily as it dissolves a keloid. But this does not take place and the amount of connective tissue usually increases with subsequent radiations.

It must be concluded then that morphological changes which take place in tumor tissue under the influence of actinic rays are two-fold. There occurs an inhibition and ultimate destruction of the tumor cells with irritation and consequent proliferation of surrounding connective tissue. But the beginning of this new connective tissue cannot be looked for in the normal tissue surrounding the tumor. The post-mortem study of the carcinoma of the sigmoid showed that there was no new connective tissue formed anywhere in the normal organs under the influence of radiation. The source of this new connective tissue must be looked for either in the stroma of the tumor or in the round cell infiltration which always closely follows the destruction of the tumor cells by the rays.

It may be stated then that the destruction of the tumor cells is the primary and the formation of new sclerotic connective tissue a secondary but at least as important a phase in the morphological changes which take place in tumors under the influence of actinic rays.

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Discussion:

Dr. Sittenfield: I would like to ask Dr. Levin whether he has studied vascular changes in the tumor and whether he has any notion as to just how the cells are influenced,—whether by dissociation of the chemical component parts of the cells or by destruction of the generative apparatus of the cell as held by Wassermann, or by inactivation of intracellular ferments?

Dr. Levin: So far as blood vessels are concerned, there takes place a condition similar to endarteritis obliterans which decreases the amount of blood supplied to the tumor causing inhibition and death of the tumor cell. As to Dr. Sittenfield's second question, it is impossible at present to determine the mechanism of the biological action of the actinic rays.

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AMYLOIDOSIS: (*a*) INCIDENCE. (*b*) REPORT OF CASE WITH EXTENSIVE AMYLOID-LIKE DEPOSITS IN ORGANS NOT SHOWING ANY OF THE CHARACTERISTIC REACTIONS

R. A. LAMBERT, M.D.

(*From the Pathological Laboratory of Montefiore Hospital and the Department of Pathology of the College of Physicians and Surgeons*)

(*a*) INCIDENCE

Among fifty-five autopsies performed at Montefiore Hospital during the past eight months nine cases of amyloidosis have been encountered, an incidence of 18 per cent. Chronic pulmonary tuberculosis was the cause of death in twenty-one cases, eight of which, or 38.1 per cent., showed a deposition of amyloid in various organs.

As will be seen from the accompanying table these figures suggest that amyloidosis is far more common in this hospital than in other institutions from which statistics on this point are available. Fortunately, we are able to compare these figures with those obtained from the records of the Presbyterian Hospital where during the past seven years only six cases of amyloidosis have been observed in approximately eight hundred and fifty autopsies, an incidence of 0.7 per cent.

Also at the College of Physicians and Surgeons, where autopsy material is received from various hospitals, only twelve cases of amyloidosis have been seen in nearly one thousand autopsies. Statistics from foreign hospitals show, too, a far lower incidence. It has seemed to us that a consideration of the possible causes for this striking difference in frequency should prove of interest.

TABLE

	Chronic Tuberculosis	Cases Showing Amyloidosis	Incidence, Per Cent.	Non-Tuberculous Cases	Cases Showing Amyloidosis	Incidence, Per Cent.	Total Autopsies	Total Number Cases of Amyloidosis	Incidence, Per Cent.
Montefiore Hospital.....	21	8	38.1	34	1	3.0	55	9	16.4
Presbyterian Hospital ..	50	2	4.0	800	4	0.5	850	6	0.7
College of Physicians and Surgeons	158	9	5.7	842	3	0.35	1,000	12	1.2
Vienna (Blum's statistics)	2,500	221	8.8	15,653	76	0.49	18,153	297	1.5
Kiel (Wicht's statistics).							2700	97	3.6

It should be stated in the beginning that the number of autopsies in the Montefiore series (fifty-five) is rather small for any conclusive statistical study, but the fact that only sixteen[1] cases of amyloidosis have been found in one thousand, eight hundred and fifty autopsies outside of Montefiore Hospital goes far to prove that the high incidence there cannot be regarded as accidental.

In discussing this problem with Dr. Wachsmann, Superintendent of Montefiore Hospital, and others, it has been suggested that the patients in that institution differ from those in other general hospitals in at least three respects: (1) Most of the patients are foreign born. This was true of all of the cases of amyloidosis;

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[1] Eighteen cases are recorded in the table but two of these were Montefiore cases, the material from which was sent to the College of Physicians and Surgeons.

(2) All patients belong to the Jewish race; (3) The tuberculous patients admitted differ in certain respects from those in many other hospitals.

Regarding the first factor, namely, the nationality of the patient, it has been stated by many who have attended autopsies in Berlin and Vienna, that amyloidosis is much more common in Europe than in America. The statistics from Vienna and Kiel (see table) seem to disprove this idea, since the percentage of amyloidosis in total number of autopsies as well as in cases where death was due to tuberculosis is not much higher than at the College of Physicians and Surgeons, and not nearly so high as in the Montefiore series.

At Vienna in calculating the percentage of tuberculous cases showing amyloid deposits, cases of acute general miliary tuberculosis and tuberculous pneumonia were probably included, whereas such cases were carefully omitted in the preparation of our statistics. We made this selection of tuberculous cases, firstly, because amyloid is practically never found in acute tuberculosis; and, secondly, because all the cases at Montefiore Hospital were chronic. Therefore a fair comparison necessitated a selection of cases of the same type at the other institutions. If we should include in our statistics from the College of Physicians and Surgeons and the Presbyterian Hospital deaths from acute tuberculosis the percentages of amyloidosis in tuberculosis would fall considerably lower than those for Vienna and Kiel.

Looking into the question of nationality of the six cases of amyloidosis at the Presbyterian Hospital, we find one Italian, one Englishman, two Hungarians, one American of German parentage, and one American negro. The percentage of foreign-born patients admitted to the hospital is about 40 per cent., but if to this group are added those of foreign parentage the percentage of "foreign" patients would probably reach 75. It is seen therefore that these figures do not give much support to the idea that foreigners are very much more susceptible to amyloid disease.

The second suggestion, that of a racial predisposition, is one upon which our available statistics are not altogether conclusive. At the College of Physicians and Surgeons no record of race has been kept. At the Presbyterian Hospital, although about 12 per cent. of the admissions are Hebrews, autopsies upon people of

Jewish faith are rare, comprising less than 5 per cent. of the total number. Thus we are able to compare the records of a hospital where all are Hebrews with those of another where there is only a small percentage of such cases. This comparison (see table), which shows at Montefiore Hospital an incidence of amyloidosis nine times as great in cases of chronic tuberculosis and six times as great in non-tuberculous cases as at the Presbyterian Hospital, certainly suggests a definite racial susceptibility. However we hesitate to form any conclusion on this point because a careful study of the cases at the two institutions, which brings us to the third possible predisposing factor, shows that the types of patients, especially those of chronic tuberculosis, admitted to the two institutions differ very materially.

The patients coming to autopsy from the tuberculosis division of Montefiore Hospital show as a rule far advanced pulmonary tuberculosis of long duration (average of those coming to autopsy, three years and four months). At the Presbyterian Hospital the cases of chronic pulmonary tuberculosis give a history usually of short duration (average of those coming to autopsy, nine months). While we have included in the fifty Presbyterian Hospital cases only those which were obviously chronic with cavity formation in lungs, or extensive bone lesions, we do not think that the comparison between these and the Montefiore cases is altogether satisfactory.

In order to settle conclusively the question of a racial susceptibility it will be necessary to obtain statistics from an institution receiving non-Jewish patients, more nearly of the type found at Montefiore Hospital.

The experimental work of Davidsohn and others, who have shown that certain strains of mice and rabbits are much more susceptible than others to the development of amyloidosis, is at least suggestive in this connection.

The tentative conclusions from this brief review of twenty-seven cases of amyloidosis seen in New York among nineteen hundred and five autopsies are as follows:

1. The influence of nationality upon the incidence of amy-

loidosis is negligible. That the condition is less common in America than in Europe is doubtful.

2. A predisposition to amyloid disease on the part of Hebrews is suggested, though not proved, by the high incidence observed at Montefiore Hospital.

3. The most important factor in determining the incidence of amyloidosis in any hospital is the type of patient admitted. Tuberculosis of long standing, involving extensive tissue destruction, is the condition most often associated with amyloidosis (70 per cent. in our cases).

(*b*) REPORT OF CASE WITH AMYLOID-LIKE DEPOSITS IN ORGANS

In eight of the nine cases of amyloidosis observed at Montefiore Hospital, and in all of the eighteen cases at the Presbyterian Hospital and the College of Physicians and Surgeons, the amyloid-containing organs showed the usual reaction with iodine. In one of the Montefiore cases, however, the reaction was entirely negative.

The case was that of a young man, twenty years old, who had shown symptoms of pulmonary tuberculosis for two years: cough, expectoration, hemoptysis, afternoon fever, etc.

At *autopsy* lungs showed extensive tuberculous lesions with numerous cavities in right lung. Spleen weighed 800 grams, was hard and on section presented the picture of a typical "sago" organ. The liver weighed 2,200 grams, was very firm and friable and showed the characteristic bacon-like translucency. In the kidneys no striking gross changes were apparent. The adrenals were rigid as though preserved in formalin.

Microscopical Examination: In the spleen, liver, adrenals and glomeruli of kidneys an extensive deposition of a hyaline pink-staining material was found, which presented the distribution characteristic of amyloid.

Application of iodine to the gross specimen, as stated above, gave no reaction. Negative results were likewise obtained with methyl-green and iodine-green. Methyl-violet gave in places a very faint reddish tinge which appeared to be more pronounced in the hyaline material in immediate proximity to the blood vessels. This faint staining was clearly seen in the liver sections. The tests were applied on fresh tissue and on specimens fixed for a short time in formalin. Some of the sections were first treated with weak acetic acid in order to neutralize any alkali present.

There seems little doubt, therefore, that we are concerned here either with deposits of an incomplete amyloid material or a general hyalinosis, a condition not recognized by those who have worked in this field. It has been clearly demonstrated by Davidsohn and others in the experimental amyloidosis of mice and rabbits that there are at least two stages in the development of amyloid. In the earlier stage the deposits do not give the usual reaction. The reaction with the methyl-violet group of dyes appears first, usually in the spleen. The iodine reaction is obtained later. Only the completely finished product, which is found rarely in organs other than the spleen and liver, gives the iodine-sulphuric acid reaction. In the present case such an extensive deposition would suggest a late rather than an early stage. It is possible, however, that there may have been a very rapid formation and deposition of the incomplete amyloid during the last few weeks of the patient's life, and that sufficient time had not elapsed for its complete development. So far as we have been able to find out no case like this one, with such extensive deposits giving none of the usual reactions, has been described.

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Discussion:

DR. FIELD: One reason that would suggest itself to me why we see so few cases of amyloidosis is that most of our material is obtained from hospitals which have an "acute" service. I imagine that more is seen on the Island in the almshouse and among their chronic cases than in Bellevue where the service is "acute." This has impressed itself on me since going down to the Neponsit Hospital, where children with bone and joint tuberculosis are treated. The number of cases in which the clinicians have diagnosed amyloidosis is comparatively large. In Louisville I saw, in my two years there, more amyloidosis than I have seen in my twelve years in New York. The cases were found among negroes who had had chronic suppurative lesions extending over periods of many years. I do not think that the suppuration is limited to any one type of organism. The only constant feature in my experience has been a suppurative process, though, of course, this generally showed many types of microorganisms.

Dr. Zinsser: I would like to ask whether amyloid change has any relation to any particular type of suppuration. Is it associated with any specific variety of microorganisms or is it the result of suppuration in general?

Dr. Larkin: We must ask what is amyloid degeneration. Chemists have not given us definite information as regards its chemical content. We are not in a position to tell the different stages of amyloid or hyaline degeneration. Neither macroscopical nor microchemical tests are absolutely proved. I would be chary of putting down such a specimen as amyloid, where the microchemical test does not confirm it. I think Dr. Zinsser's question is a pertinent one as to what are the factors that produce it. We look for amyloid degeneration in chronic suppurative conditions, bone tuberculosis, psoas abscess, and so on. There are a number of suppurative conditions of which we do not know the character, which do not produce amyloid. We must differentiate these cases from those giving the microchemical test. This specimen does not represent amyloid conditions *per se*. It is one of the intermediate groups.

Dr. Lambert: Replying first to Dr. Zinsser's question: in the experimental production of amyloid it has been shown that almost any pyogenic organism may be used. Killed cultures are quite efficacious.

Regarding Dr. Field's last statement, I think that the common association of amyloid disease with tuberculosis is too well established to warrant discussion. In Blum's series at Vienna (two hundred and ninety-seven cases in eighteen thousand one hundred and fifty-three autopsies) tuberculosis was the etiologic factor in 79 per cent.: in our series, 70 per cent.

With Dr. Field's other remarks I am in thorough accord. His experience makes me feel certain that the high incidence at Montefiore Hospital is probably to be attributed to the very chronic type of tuberculosis treated there.

REPORT ON TWO FORMS OF CARDIAC ABNORMALITY

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The study of the two hearts which form the basis of this report is presented more with the idea of placing the results on record than of giving in connection with it any extended discussion of the anatomical or pathological features of the organs. The material was obtained from the Pathological Laboratory of the New York Nursery and Child's Hospital. During the past year we have met with almost all the common types of cardiac abnormality and in addition we have found three conditions of more than usual interest:

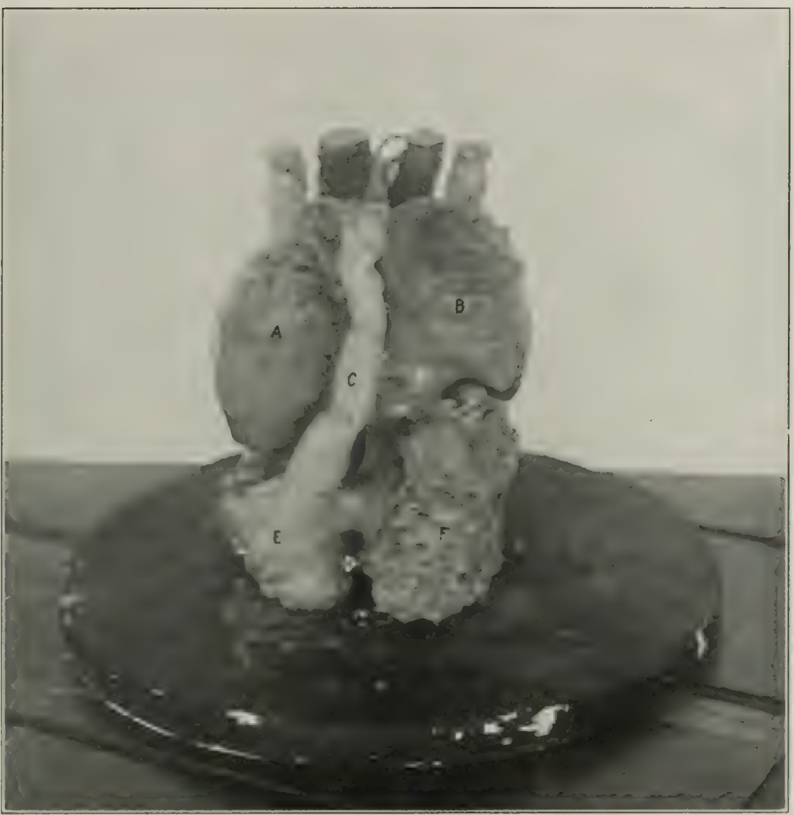
(*a*) Complete transposition of the arterial trunks.
(*b*) Complete absence of the interventricular septum.
(*c*) Aortic atresia and rudimentary left ventricle.

The first two will be shown at this meeting; the third will be made the subject of a separate report at some later date.

COMPLETE TRANSPOSITION OF THE ARTERIAL TRUNKS

CASE I. *Pathology.*—In this case, we find the ventricular portion of the heart to be normal in all its features; position, size, and composition of its costo-chondral surface. The heart is normally divided by its interventricular groove. The right ventricle externally appears somewhat larger than the left and forms the apex of the heart, but upon opening the organ, we find that the cavity of the left ventricle is larger than that of the right. The walls of the right ventricle are thicker than those of the left. The auriculo-ventricular valves are thin, delicate, and present extra flaps; the right auriculo-ventricular orifice being guarded by a valve which presents four flaps, the left auriculo-ventricular orifice by a valve with three flaps. The auricles are normally formed and separated from one another and there are no defects in the interauricular septum. The foramen ovale is still patent along its anterior border.

The great vessels present a condition of complete transposition, the aorta arising from the right ventricle, the pulmonary artery from the left. They emerge from the heart in an almost directly transverse plane close together and near the anterior aspect of the heart. They both pass upward and somewhat backward. The aorta then sweeps to the left and posteriorly over the bifurcation of the pulmonary artery, giving off its three great vessels from its superior aspect. The inferior aspect of the vessel receives the ductus arteriosus which is comparatively long, twisted, and patent. The aorta then passes over the root of the left lung. The pulmonary artery passes upward and backward under cover of the aorta and divides into three vessels, the ductus arteriosus, which arises slightly to the left of the bifurcation, and the two pulmonary arteries. The coronary arteries are derived from the



Photographs of reconstructions of the cardiac apparatus in three cat embryos—7, 9, 11 mm.—in length, showing the effect of torsion on the truncus arteriosus and the division of that vessel into the aorta and the pulmonary artery.

FIG. 1. 7 mm. cat embryo. *a*, right auricle; *b*, left auricle; *c*, common arterial trunk; *d*, interventricular septum; *e*, right ventricle; *f*, left ventricle.

aorta. The semilunar valves are normal both in number and arrangement as are also the pulmonary veins.

In this case we have an arrest of torsion of the great vessels whereby the orifice of the aorta remains on the right side, that of the pulmonary artery on the left. The interventricular septum, rising from below and coming in contact with the septum trunci, establishes connection between the aorta and the right ventricle, and the pulmonary artery and the left ventricle. The difficult thing to reconcile with this course of events is that in this case

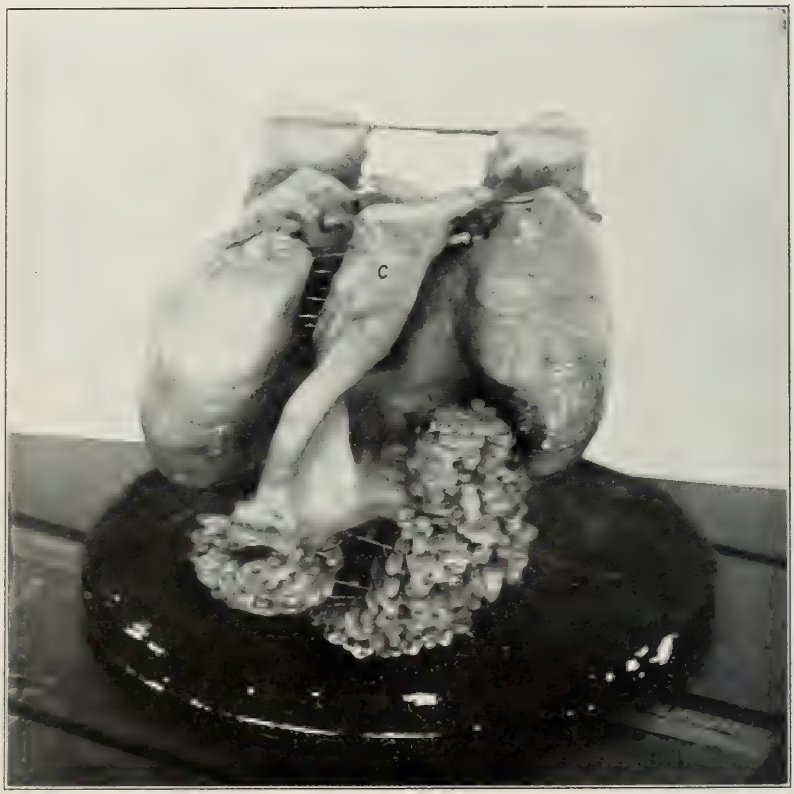


FIG. 2. 9 mm. cat embryo. *c*, common arterial trunk. The truncus shows here the effect of the torsion.

the ventricles have obeyed the tendency to rotate and have taken up their normal position, whereas the truncus which is supposed to obey the same factors in its rotation has completely ignored these factors and has remained unrotated.

Transposition of the Arterial Trunks.—According to the teachings of Rokitansky, transposition of the great vessels, *Rechtslage* of the aorta, as well as certain types of developmental stenosis of the aorta and pulmonary artery, are due to irregularities in the formation of the septum within the aortic bulb and to its malunion with the interventricular septum.

Types.—(*a*) Complete transposition in which the vessels arise from the ventricle to which they do not normally belong.

(*b*) Corrected transposition in which the vessels arise from the proper ventricle but present an inverted relationship to each other.

(*c*) Partial transposition.

In studying these conditions it is necessary for us to keep in mind the processes through which the heart passes in its development in order that we may understand the anomalies which arise through arrest of development or perverted development. In order that these factors may be clear in our minds, it may be well for us to review the steps in the development of these structures.

As will be remembered, the primitive heart tube in its growth becomes twisted upon itself in the shape of the letter S, the venous end being situated posteriorly and to the left in the position where the auricles will develop; in front of the venous end of the heart tube lies the ventricular portion and passing upward mesially and cephalad from the ventricular portion lies the common arterial trunk. There are two fixed points to be considered in order that we may understand the process of torsion which is to determine the picture in the truncus; the first, where the venous end of the heart enters the pericardial sac; the second, where the arterial trunks leave the pericardial sac. Continued growth within the pericardial sac along the direction already initiated by the S-shaped tube produces a torsion of 180 degrees of the common truncus by which the relationship of the adult aorta and the pulmonary artery is produced; this torsion is from the left to the right. By the time that this torsion has begun to make itself felt, the process of separation between the aorta and pulmonary artery has been initiated. This separation is produced by the appearance within the cavity of the truncus of two sets of ridges which according to their relationship with the ventricle, are called proximal and distal. The proximal set of two may be called ridges *A* and *B*, and a distal set of four, ridges 1, 2, 3 and 4. The result of the external forces of rotation or torsion on these ridges is the assumption by them of a spiral twist as they pass toward the heart along the interior of the tube. This spiral twist takes a clockwise direction from above downward and to the right; on the exterior of the tube can be found furrows which correspond to the internal ridges.

We will now follow in some detail the origin of the two sets of ridges and their behavior in dividing the tube into the two large vessels. The ridges arise on sides of the tube opposite one another; the ridges *A* and *B* in the lower half, the ridges 1, 2, 3 and 4 in the upper half, of the truncus. They are so arranged that as they spiral downward in a clockwise direction one ridge of the proximal set meets and fuses with one ridge of the distal set on either side of the tube. We have thus in the composition of each ridge three parts; one horn of the septum aorto-pulmonalis (will be described in detail later); one of the ridges 1 and 3 and one of the ridges *A* and *B*. Ridges 1 and 3 are large, 2 and 4 are small.

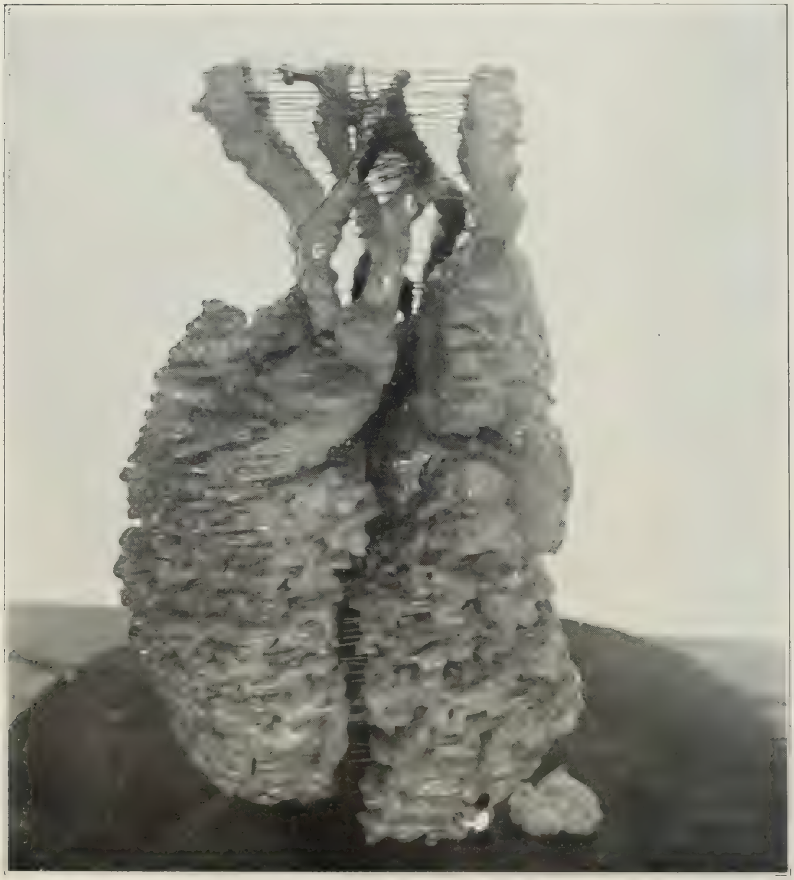


FIG. 3. 11 mm. cat embryo. *g*, aorta; *k*, pulmonary artery; *i*, ductus arteriosus. Complete separation of the truncus into the aorta and the pulmonary artery.

Prior to this there has been a separation of the fourth and sixth aortic arches external to the pericardial sac into what will later become the adult aorta and pulmonary artery. This so-called septum aorto-pulmonalis now invades the portion of the truncus which lies within the pericardial sac and adds itself to the factors producing a separation between the two vessels. We now have the two sets of ridges, *A* and *B*, and 1, 2, 3 and 4, and the septum aorto-pulmonalis. This septum pushes forward its two horns which thus enclose between them a free sickle-shaped edge and finally effects a junction with ridges 1 and 3, which in turn becomes continuous with ridges *A* and *B*. The process thus initiated progresses slowly, the sickle-shaped free margin passes caudally. The ridges become thicker and thicker until they fuse from above downward and the two vessels become completely separated. Through the spiral arrangement of the ridges we now have two curving tubes which looked at from above present a pulmonary artery passing forward and to the right, and an aorta passing posteriorly and to the left closely applied and wrapped about one another.

At a somewhat later time the vessels become independent through their separation by the external furrows which corresponded to the internal ridges. Having now seen the single trunk divided into two separate vessels, we can follow the development of the semilunar valves. Through the fusion of ridges 1 and 3 each vessel contains one half of each fused ridge and in addition the smaller ridge which lies between the larger ridges. The aorta contains ridge 2, the pulmonary artery ridge 4; these smaller ridges fade away distally but in the region of the junction with the ventricle they enlarge and become hollowed out, forming one of the semilunar valves; the other two valves are formed through similar processes taking place in the halves of ridges 1 and 3 left in each vessel.

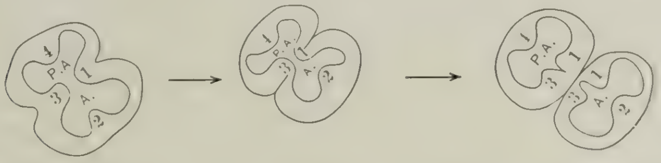
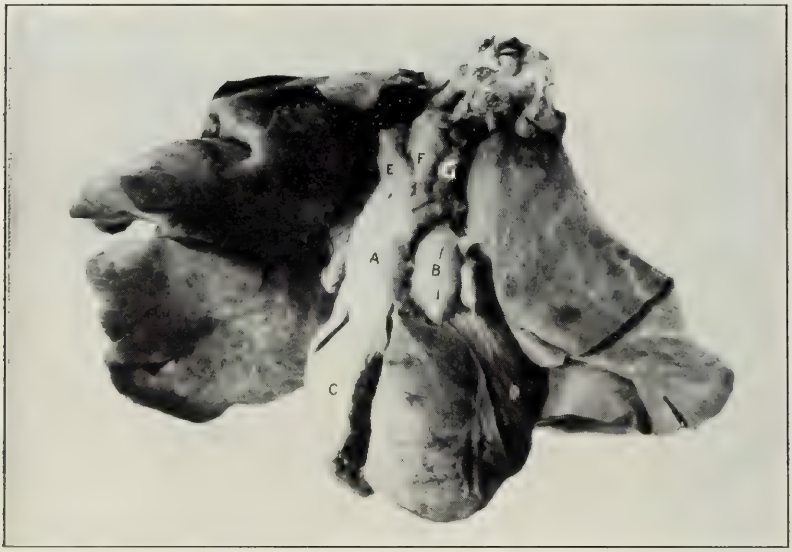


Diagram to illustrate the formation of the semilunar valves which develop by the hollowing out of the lowermost extremities of the ridges 1, 2, 3 and 4. The diagram also illustrates how ridges 1 and 3 participate in the formation of valve-flaps in both the aorta and pulmonary artery.

The two vessels now open into the ventricle which has become divided by the interventricular septum into the smaller right and the larger left half. This septum grows up from the floor and both anterior and posterior walls at the same time so that it presents two horns, an anterior and a posterior, containing between them a free sickle-shaped border. Meanwhile, the most inferior portions of ridges *A* and *B* have grown forward, joined, and have produced what we call the proximal bulb septum. Viewing this from above, we find that it passes from the right superiorly and posteriorly toward the left anteriorly and inferiorly, and thus sets itself in a warped fashion on the interventricular septum which is growing upward to fuse with it.

The anterior cornu of the septum trunci fuses with the anterior horn of the interventricular septum, while the posterior cornu of the septum trunci spreads out on the posterior ventricular wall and fuses with the right extremity of the posterior endocardial cushion of the atrio-ventricular canal. If we follow the boundaries of the interventricular foramen, we now find them as follows: starting from above and behind we have the warped under surface of the septum trunci leading to the anterior cornu of the interventricular septum, advancing downward along the anterior wall to the inferior sickle-shaped intercornual margin, back and finally upward along the posterior cornu of the septum to the right extremity of the posterior endocardial cushion. This space is gradually closed in by downgrowth of the septum trunci and upgrowth of the interventricular septum until they meet and fuse.



Photographs of the specimens embodied in the report.

FIG. 4. Complete transposition of the arterial trunks. *a,* aorta; *b,* pulmonary artery; *c,* right ventricle; *d,* left ventricle; *e,* innominate artery; *f,* carotid artery left; *g,* left subclavian.

As a result of the direction of the septum trunci toward the right end of the posterior endocardial cushion, we find the right atrio-ventricular opening close to the septum, the left separated from it by some distance.

Having seen the mechanism by which the great vessels become separated from one another, it is a comparatively easy matter to see how readily anomalies of the arrangement of the great vessels may occur. Rokitansky, in his schema of the rotation of the great vessels, distinguishes two main groups according to whether the transposition was corrected or not with eight subdivisions in each group, the subdivisions being stages in the spiral twist and the form that the transposition would take in each subdivision were the spiral arrested at that point. In his schema *A* we find the transposition is corrected, for, in spite of the altered relation between the two great trunks, they are connected with the proper ventricle by a compensatory twist of the interventricular septum. In schema *B* we find, however, a true transposition of the trunks, there having been no correction and the trunks being connected with the wrong ventricle. The behavior of the interventricular septum determines whether correction takes place or not.

Partial Transposition.—This is relatively infrequent, but it exists and results in the large vessels arising from the same or a common ventricle and is due to the application of the interventricular septum to either one side or the other of the large vessels instead of between them. In such cases it is the rule to find the orifice of one or the other of the vessels encroached upon.

An anomalous septum may cut off an abnormal cavity as an origin for the two vessels. Unless some other anomaly is present and persists, complete transposition is incompatible with life, for we have the lesser circulation cut off and transformed into a closed circulation. This deprives the blood of any opportunity to gain access to the greater circulation unless it is by some abnormal channel as a patent ductus, a patent foramen ovale or a defect in the interventricular septum; correspondingly, the blood in the greater circulation fails to gain access to the lungs.

Defects of the Interventricular Septum.—As is usually the case with cardiac lesions, we often find other lesions associated with defects in the interventricular septum, the great vessels being often affected. This is readily understandable when we consider the close relationship between the two sets of structures.

Types: (*a*) Complete.
(*b*) Incomplete.

If complete we may find either a condition known as cor triloculare biatriatum or cor biloculare, depending upon the presence or absence of the interauricular septum. In association with the latter we may find a common auriculo-ventricular orifice and an undivided truncus.

These defects may be viewed mainly as arrests of development and in the majority of cases may be explained by a simple schema of the development of the interventricular septum. The septum develops from four component parts:

(*a*) The proximal septum trunci.
(*b*) The anterior horn of the interventricular septum.
(*c*) The posterior horn of the interventricular septum.
(*d*) The intercornual septum.

(*a*) Defects in the septum due to faulty development of the septum trunci constitute the most frequent type. The defect is located close under the orifices of the great vessels and occupies the area known as the "undefended space" or the pars membranacea.

(*b*) Defects due to the failure to develop of the anterior horn of the interventricular septum are located in front of the pars membranacea.

(*c*) Defects due to failure to develop in the posterior horn are correspondingly located behind the pars membranacea.

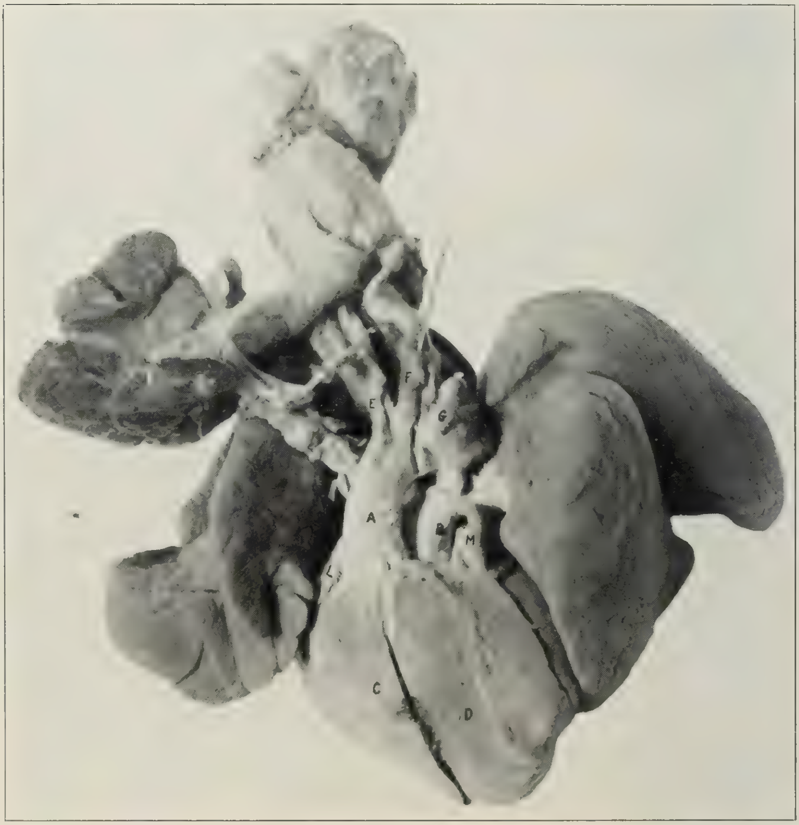


FIG. 5. Complete transposition of the arterial trunks. *a*, aorta; *b*, pulmonary artery; *c*, right ventricle; *d*, left ventricle; *e*, innominate artery; *f*, left carotid artery; *g*, left subclavian; *k*, ductus arteriosus; *i*, thymus; *l*, right auricle; *m*, left auricle.

(*d*) Defects due to faulty development of the intercornual portion may lie at any point in the septum, but they are more usually close up under the pars membranacea and are due to failure of the two septa to join.

COMPLETE ABSENCE OF THE INTERVENTRICULAR SEPTUM

Case II. *Pathology.*—Externally the heart is quite normal in shape. It weighs 170 grams, measures 8 × 7.5 × 6 cm., and has a fairly well defined and formed apex. There is, however, no interventricular groove to be seen and no indication of any division into two ventricles. The great vessels appear to arise from the heart on almost the same plane; the aorta from the right side, the pulmonary artery from the left and slightly posterior so that we have here also a case of transposition of the vessels. The vessels are cut off so close to the heart that their further course cannot be followed but so far as we can see there is no indication of any ductus. Upon opening the auricles, we find that the right auricle is the larger, the right auriculo-ventricular orifice measuring 5 cm. in circumference; the left auricle is smaller, its auriculo-

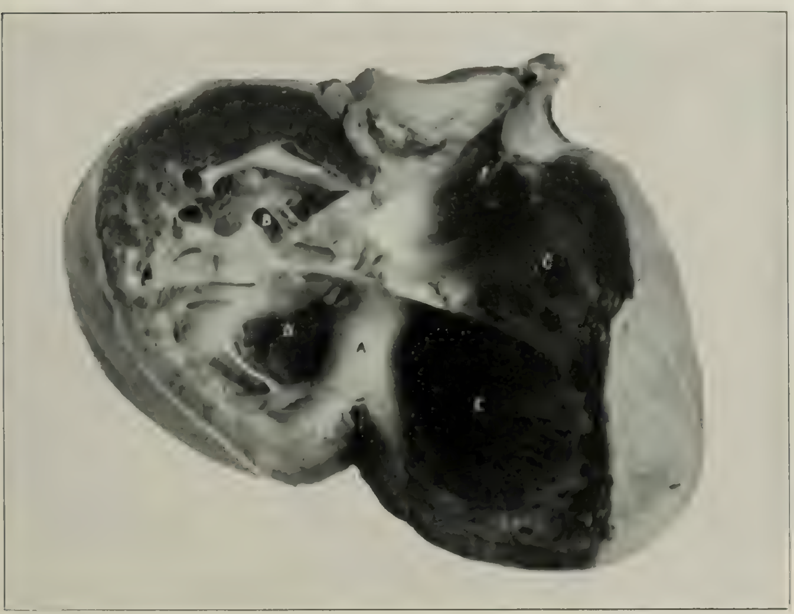


Fig. 6. Cor triloculare biatriatum. *a*, septum trunci; *b*, left auricle; *c*, right auricle; *d*, left ventricular portion of common ventricle; *e*, right ventricular portion of common ventricle.

ventricular orifice being 3 cm. in circumference. A rough estimate of the capacities of the two auricles would make that of the right three times that of the left. The right valve presents three flaps and several thickenings varying from 1 to 6 mm. in size. The left valve presents two main flaps which are broken up into a number of smaller ones by the insertion of anomalous chordæ tendineæ. The interauricular septum forms a common site of origin for both valves and a common papillary muscle receives chordæ from each of these valves.

There is but a single ventricle without any evidence of attempt at division in two by an interventricular septum. The walls are thick and heavy and present large papillary muscles and columnæ carneæ. The auriculo-ventricular orifices present at the posterior part of the ventricle, the orifices of the great vessels at the anterior part of the ventricle, the aortic on the right, the pulmonary on the left. These orifices are separated by a thick, heavy band of muscle fibers passing anteriorly and posteriorly between them and represent the proximal septum trunci.

I am much indebted to the anatomical department of the College of Physicians and Surgeons for permission to reproduce the reconstructions indicating the divisions of the truncus into the aorta and the pulmonary artery.

ADDITIONAL CASE REPORT

Since the report of the preceding case of complete transposition of the arterial trunks, it has been our good fortune to find another instance of the same pathological condition. The two hearts are almost identical and present pictures that agree in every essential point. In the second instance the child lived for about six hours after birth. There was a great deal of cyanosis affecting particularly the extremities, the hands and feet being a deep indigo.

Upon opening the thorax the organs were found to be intensely engorged and congested, the lungs being almost black. As soon as the pericardium was opened, the transposition was recognized and the organs were removed *in toto*. As in the previous case the aorta arises from the right ventricle, passes upward, giving off the great vessels from its convexity and receiving on its concavity the patent ductus arteriosus as it passes over the pulmonary artery. The pulmonary artery shows the same arrangement as we have seen previously, dividing into its two pulmonary arteries under cover of the aortic arch, the left one in turn giving off immediately the ductus arteriosus.

Upon opening the right auricle, it is found to be large and dilated; the foramen ovale is patent along its anterior edge. The left auricle is considerably smaller than the right. The right ventricle presents the origin of the aorta. Passing posteriorly and slightly to the right behind the origin of the aorta is a thick heavy band of muscle fibers which represents the lower margin of the primitive septum trunci. It serves to separate the aorta and the right auricle and from its posterior aspect it gives origin to one of the flaps of the tricuspid valve. The origin of the two coronary arteries can be seen in the posterior and left sinuses of Valsalva of the aorta. The flaps of the mitral and tricuspid valves are normal in structure and number. The left ventricle presents the origin of the pulmonary artery arising anteriorly and close to the interventricular septum. The interventricular septum presents no defects.

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Discussion:

Dr. MacCallum: I think Dr. Riley has covered the ground pretty thoroughly. There is one striking point in this connection and that is the mathematical regularity with which certain principles are carried out in transpositions of the great vessels. In making out his schema Rokitansky was able to prophesy anomalies of the great vessels and several types which had not been reported prior to this, but which he had figured as possible in his schema were later reported by different observers. A good deal of light can be thrown on this subject by cutting the hearts transversely close under the great vessels to show the disposition of the aortic and interventricular septa and by comparing them with pathological hearts cut in a similar fashion. A better idea of the relationships can be gathered in this fashion than by cutting the heart in the usual fashion through the valve flaps.

Dr. Larkin: I have had rather an interesting anomaly of the heart recently. The child had spina bifida., In these cases anomalies of the cardia are not infrequent. The child presented several abnormal conditions. This abnormality of the heart has never before been described. The charts of Dr. Riley give an idea of the disposition of the vessels. In my case the aorta leads directly into the right and also into the left ventricle, the pulmonary artery being normal. The aorta is normal until the base of the heart, where there is a lack of the septum. The aorta is bifurcating, one part goes to the right ventricle, one to the left.

Dr. Riley: The explanation is I think that the interventricular septum comes up from below and the septum trunci comes from above; they do not meet and the interventricular septum separates the aorta into two parts.

Dr. Larkin: There is no evidence of any upper septum at all.

Dr. Riley: You have a division of the truncus into two vessels.

Dr. Larkin: The pulmonary goes into the right ventricle, the aorta into the left. There is no abnormality of the pulmonary.

FIVE CASES OF PRIMARY CARCINOMA OF THE APPENDIX

A. B. EISENBREY, M.D.

(From the Pathological Department of St. Luke's Hospital, F. C. Wood, Director)

The five cases of primary carcinoma of the appendix to be reported were found among approximately eighteen hundred appendices examined in the pathological laboratory of St. Luke's Hospital during the past three years. The incidence of this lesion among our cases is thus seen to be 0.3 per cent. as contrasted with the 0.4 per cent. and 0.5 per cent. reported by other observers. Four of the five cases were females and the ages of the patients varied from nineteen to sixty years. In one of the cases the lesion was found at autopsy, and in but one of the remaining four cases was attention directed to the appendix before operation. In two instances only was the presence of carcinoma suspected from the gross appearance. In two of the appendices the lesion was situated in definite thickened areas at about the mid-point in the organ; in two others it was found in the enlarged clubbed tip, and in the fifth in the constricted fibrosed tip. In none of the cases was there macroscopical evidence of extension through the serous coat of the organ, nor was the organ adherent to the surrounding structures; no other carcinomatous lesion was found elsewhere in the body, either at operation or at autopsy. The appendices ranged from 4.5 cm. to 6 cm. in length and from 0.5 cm. to 1 cm. in diameter, except at the site of the lesions, of which the largest was 1.2 cm. in diameter.

Microscopically the lesions all showed the most frequently described type of appendiceal carcinoma: destruction and replacement of glandular and lymphoid structures by a rather dense fibrous stroma, supporting islands and strands of cells of the spheroidal epithelial type which also invaded the muscularis and in several of the cases were found in the subserous structures. In two of the cases the cells were smaller, fewer large islands were found and the growth in general was of a more scirrhus type.

(We had a sixth case of carcinoma of the appendix which is not included in this report. It was of the gelatinous type and while the chief growth and origin appeared to be in the appendix the cæcum was also extensively involved so that it is not justifiable to class it as primary in the appendix.)

Case I. (Path. No. 8084.) Female, aged twenty-five, gave a history of pelvic disturbances following birth of child six and a half years before but showed no symptoms referable to the appendix. At operation for the correction of retroversion of the uterus the appendix was found to be constricted at the distal fifth of its length and was removed. The carcinomatous growth was seen, in longitudinal section of the constricted portion, to involve the area nearest the base where its relation to the mucosa was well shown. The region of the tip was free from the growth. The islands of cells in this case were large with rather large and vesicular cells.

Case II. (Path. No. 8415.) Male, aged sixty, was operated upon for acute appendicitis of three days duration. He gave no history of any previous disturbances of like nature. At operation the appendix gave evidences of a chronic inflammatory process with an added acute congestion. The middle portion of the organ was slightly thickened and densely fibrosed. The cells were of a smaller type, the islands small but numerous, with a well marked invasion of muscularis and subserosa.

Case III. (Path. No. 10845.) Female, aged thirty-one, gave a history of symptoms referable to the pelvic organs, extending over a period of five years. No symptoms were directly referable to the appendix. At operation for double salpingitis and retroverted uterus the appendix was found thickened throughout, the serosa was congested but smooth, and there was considerable clubbing of the tip. In this case the cells were of the large clear type, the cell nests large, and the invasion of the subserosa most marked.

Case IV. (Path. No. 11554.) Female, aged forty-two, was operated upon for fibromyomata of the uterus and the appendix removed incidentally. There was marked clubbing of the tip and considerable congestion and a slight exudate on the serosa over it. The lesion was well confined to the region of the mucosa and submucosa. The cells and cell-groups were of the large type.

Case V. (Autopsy No. 1153.) Female, aged nineteen, died of septicæmia following a septic endocarditis of probable rheumatic origin. The appendix appeared normal externally except for a hard thickening about 1 cm. in diameter at its middle. On section this was of dense fibrous structure with a rather granular surface of yellowish color, an appearance noted in the other cases of this series which we believe characteristic of this type of lesion. In this case the growth was of the scirrhus type, the cells were small and the invasion to the serosa well marked.

Discussion:

Dr. Ewing: In connection with reports of cases of carcinoma of the appendix it has been objected that while these processes have the morphology of carcinoma they are not genuine malignant tumors, but merely atypical overgrowth of aberrant epithelial rests or of displaced portions of mucosa which possess a very limited power of proliferation. Many of the cases fail to show the clinical features of a malignant tumor and it may well be that some of the tumors are practically at a standstill when observed. I think, however, that they are properly regarded as true carcinomas of their own peculiar sort. We have no right to demand that they should show all the characteristics of malignant tumors in other parts of the body. They most resemble the basal cell carcinomas.

Dr. Eisenbrey: There are mitotic figures in these specimens, but they are hard to find. In none of the cases were there enlarged glands of the mesoappendix, nor was there any demonstrable carcinoma elsewhere, except in the case of gelatinous carcinoma, where the cæcum was also involved. That case is not included in this series.

Dr. Pappenheimer: Are there any cases in the literature in which widespread metastases have been reported?

Dr. Eisenbrey: Yes, there are some cases where there is active recurrence. So far as this particular type goes, I do not believe there are any recurrences.

EXTRA-MEDULLARY MYELOID CHANGES IN ORGANS

W. G. MACCALLUM, M.D.

Dr. MacCallum presented preparations from two cases to illustrate the appearance of myeloid tissue in organs other than the bone marrow. One of these was a case of so-called osteosclerotic anæmia in which the destruction of practically all of the bone marrow by metastases from a carcinoma of the prostate resulted in an extreme anæmia and the spleen and liver were found laden with myeloid cells. The venules of the spleen were especially filled with myelocytes. The second case was one of myeloid leukæmia in which distinct nodules of myeloid tissue including megalocaryocytes were found in the liver. Two views have been held as to the origin of these cells; one being that elements originally present in most tissues and capable of production of cells of the blood are reformed by a sort of reversion to embryonic conditions; the other being that myeloid cells are swept out of the bone marrow and in case of necessity colonize in other organs and there produce the elements of the blood. In the case demonstrated the latter view seems most probable.

Discussion:

Dr. Pappenheimer: I would like to ask whether, in the case of extreme sclerotic anemia, any myeloid cells found their way into the circulation.

Dr. MacCallum: There were foci of the same sort. I don't believe any went into the circulating blood. There were some in the adrenals.

Dr. Field: These myeloid changes are very frequently found in areas of arterio-sclerosis in the aorta, in cases which have shown only slight anemia. It would seem to me that Maximow's *Wanderzellen* can give rise to both series of blood cells when the nutritive and pressure conditions are favorable, in the same way as they do in embryonal life.

Dr. Ewing: Does Dr. MacCallum know of any definite instance in pathology where an embryonal function or tissue, once passed through its normal course of development to the adult, experiences a reversion to the embryonal condition?

Dr. MacCallum was glad that Dr. Ewing expressed himself as opposed to the idea of a reversion of cells to embryonic form and function, since he had seen no evidence of it. The explanation is difficult in the case of bone marrow formed in necrotic tissue found in the walls of blood vessels and the lung, but in other cases the theory of colonization seems better supported.

LATE RESULTS IN ACTIVE IMMUNIZATION WITH DIPHTHERIA TOXIN–ANTITOXIN AND WITH TOXIN–ANTITOXIN COMBINED WITH DIPHTHERIA BACILLI

WM. H. PARK, M.D., AND ABRAHAM ZINGHER, M.D.

(*From the Research Laboratory and the Willard Parker Hospital, Department of Health, New York City*)

The property of diphtheria toxin-antitoxin mixtures to produce immunity in animals has been known for a number of years, but its application to human beings was not practised until Von Behring recommended it in May, 1913. The early reports of Von Behring and his co-workers were so favorable that we determined to make a similar attempt at the Willard Parker Hospital.

Several series of patients were injected with mixtures of diphtheria toxin and antitoxin, in such proportion that the mixture was either overneutralized, just neutral or slightly toxic to the guinea-pig. In the last series of cases (Group III) we used a mixture consisting of 85 per cent. of the L_+ dose of toxin to each unit of antitoxin, to which was added a vaccine made from killed diphtheria bacilli.

In a previous communication we published the following conclusions, derived from the results obtained in the immunization of two groups of scarlet fever patients who were injected with toxin-antitoxin:

1. Those who had a natural antitoxic immunity produced a decided increase in the amount of antitoxin in a short time (seven days), even after a single injection of toxin-antitoxin.

2. Those who had no natural antitoxic immunity were quite resistant to active immunization. Only 25–30 per cent. showed decided antitoxin production within four to six weeks after injections of toxin-antitoxin. A larger proportion produced a trace of antitoxin, and gave a fainter Schick reaction than in the original test. The reaction was still positive, however, and they were not considered immune.

3. We concluded, therefore, that toxin-antitoxin could be used

where no immediate danger of infection was present. Non-immune individuals exposed to diphtheria, however, should be protected with antitoxin.

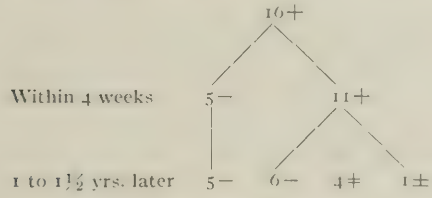
Observations on our chronic tube cases, who developed slowly, but almost uniformly, an antitoxic immunity, led us to retest with the Schick reaction those who were at first partially or totally refractory to immunization with toxin-antitoxin.

From Group II (toxin-antitoxin) we retested sixteen cases, from Group III (toxin-antitoxin plus bacterial vaccines) fifty cases, at intervals of from two months to one and one-half years after the immunizing injections.

In Group II, a series of ninety patients was originally injected who had no natural immunity, as shown by blood examination for antitoxin and by a positive Schick reaction. Of the ninety, twenty responded to the injection of toxin-antitoxin with the production of sufficient antitoxin to protect against diphtheria; seventy failed to respond to this degree, though some gave a fainter Schick reaction than they had shown before the immunization. Among the twenty no cases of diphtheria developed; of the seventy who produced very little or no antitoxin, seventeen developed clinical diphtheria twelve days and more after the first injection.

From Group II, sixteen cases were subsequently retested one to one and one-half years after the injections of toxin-antitoxin.

EARLY AND LATE SCHICK TESTS IN GROUP II.



— indicates negative Schick reaction.
ǂ indicates faint Schick reaction.
± indicates moderate Schick reaction.
+ indicates fully developed Schick reaction.

Five of the sixteen who had shown an early and decided production of antitoxin, again gave a negative Schick reaction. Of the eleven who had failed to respond at first to the immunizing injections, six now gave a negative, four a very faint and only one a positive Schick reaction.

Group III consisted of one hundred and fifty-eight patients, who had originally given a positive Schick reaction. These patients were immunized with a mixture of toxin-antitoxin and a vaccine made of killed diphtheria bacilli. Three to four subcutaneous injections were given at intervals of three to four days, each injection consisting of 1 c.c. toxin-antitoxin (85 per cent. L_+ dose of toxin to each unit of antitoxin), and 1,000,000,000 bacteria. One c.c. of the mixture injected subcutaneously into a guinea-pig produced a slight local induration and paralysis in ten days.

EARLY RESULTS IN GROUP III

158 cases

No antitoxin (+ Schick) before immunization

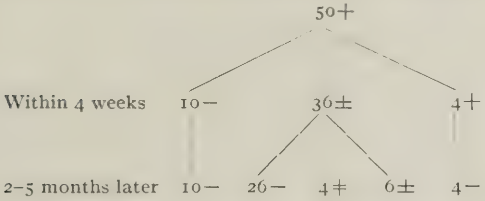
158+ (no antitoxin)

Within 4 weeks	56−	9∓	83±	10+
Developed diphtheria	0	0	0	4

Of the one hundred and fifty-eight injected patients, fifty-six or 35.5 per cent. produced enough antitoxin within four weeks to give a negative, and nine, or 6 per cent., a very faint Schick reaction. Of those who were partly or totally refractory, eighty-three, or 52 per cent., gave a moderately positive, and ten, or 6.5 per cent., a strongly positive Schick reaction. Of the ten, four developed clinical diphtheria.

To note any late development of immunity, we retested from two to five months after the injections, fifty cases of this group.

EARLY AND LATE SCHICK TESTS IN GROUP III.

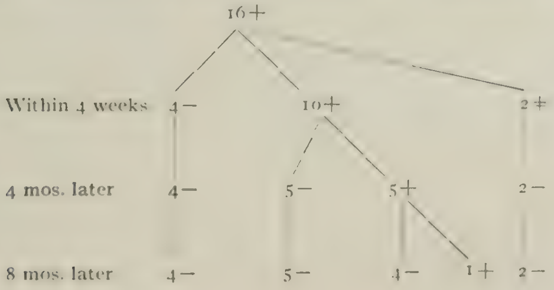


Ten of the patients who had given a definite early production of antitoxin continued to show a negative Schick reaction. Of the thirty-six patients who had produced only a trace of antitoxin, not sufficient to inhibit the Schick reaction, twenty-six now gave a negative, four a very faint and six a moderately positive reaction. The remaining four who were refractory at first, now gave a negative Schick reaction.

Grouping together the early and late successful results in this series of cases, we find that forty out of fifty susceptible individuals had developed an antitoxic immunity after the injections of toxin-antitoxin plus vaccine. The remaining ten gave a reaction which was moderately positive in six and faintly positive in four cases. These cases probably had developed some bactericidal immunity from the bacilli injected.

In addition to these, we also immunized with toxin-antitoxin, a series of sixteen normal children (Group IV), who had given a positive Schick reaction. These children were injected about a year ago, and retested with the Schick reaction four and eight months after the immunization.

EARLY AND LATE SCHICK TESTS IN GROUP IV (NORMAL CHILDREN)



Within four weeks after the injections, only four, or 25 per cent., gave a negative Schick test. Four and eight months later, these four still gave a negative reaction, thus indicating a probable lasting immunity. Ten children, who were refractory at first, now showed the following interesting results: at the end of four months, five gave a negative and five a positive Schick reaction. At the end of eight months, nine showed a negative reaction and only one was still positive. Two of the sixteen children who had given a partial early response, were found immune at the end of four and eight months.

As control, we had a group of six children who had received antitoxin only a year ago. At the end of eight months, five of the children still gave a positive Schick reaction, and one only had now become negative.

Another group that may serve as control consisted of sixty children who had received vaccines made only from diphtheria bacilli. Retested from five to eight months after the injections, forty gave a positive and twenty a negative Schick reaction.

Our results in active immunization up to the present time have led us to the conclusion that, while the toxin-antitoxin injections cannot be depended on to prevent diphtheria in the presence of immediate danger of infection because of the slow development of immunity in a majority of the cases, yet the injection is of value in rendering a community or school population immune to the danger of infection from future exposure up to a period of possibly several years.

CONCLUSIONS

1. Individuals who before treatment give a negative Schick reaction are immune probably for life, and therefore it is not necessary to inject them either with antitoxin or toxin-antitoxin.

2. Those who give a positive Schick reaction and are exposed to diphtheria and in immediate danger, should receive either antitoxin alone, or if a longer protection is desired, both antitoxin and toxin-antitoxin.

3. For general prophylaxis against diphtheria excluding im-

mediate contacts, a mixture of toxin-antitoxin alone (85–90 per cent. of the L. dose of toxin to each unit of antitoxin) or toxin-antitoxin plus vaccine of killed diphtheria bacilli, is recommended. The dose is 1 c.c. of toxin-antitoxin and 1,000,000,000 bacteria injected subcutaneously and repeated three times at intervals of six or seven days. Sufficient time has not yet elapsed to judge the value of adding the injections of the bacilli to the toxin-antitoxin.

4. The early and the late results of active immunization should be determined with the Schick test. Early results are those obtained by the application of the test within four weeks, late results from four months to two years after the immunizing injections.

Discussion:

Dr. Field: Dr. Zingher's paper is extremely interesting in that it gives us an explanation from the laboratory as to why diphtheria is a disease of the younger years and is comparatively rare among adults. When we realize that 5 per cent. of all school children harbor the diphtheria bacillus in their throats, it is readily understood why such a large proportion should be found to be immune by the Schick test.

Dr. Zinsser: The antitoxin contents of the blood are relatively high during the first year of life, then lower, again increased toward adult life, falling again in old age.

Dr. Field: It falls in with the Schick test.

Dr. Zinsser: An interesting point in Drs. Park and Zingher's paper is that they are using mixtures which are not over-neutralized, namely, an L. dose with one unit. In Behring's experiments I believe the mixtures were considerably over-neutralized.

Dr. Field: In regard to the production of antibodies by mixtures of toxin and antitoxin, I would like to call the attention of the Society to the fact that Dr. Park reported on this point before the Society fourteen years ago. The work had been done two or three years previous to his making the report, and when Ehrlich visited the Research Laboratories he was surprised to discover this point; I well remember his animated conversation with Dr. Park in regard to it.

Dr. Zingher: Antitoxin production is abundant in the horse after injections of neutralized or over-neutralized mixtures of diphtheria toxin and antitoxin. Similar results are obtained in human beings who are naturally immune. There is probably a dissociation of the toxin-antitoxin mixture *in vivo*, and a ready response of the tissue cells to the antigen. It is important to have the right proportion of toxin and antitoxin in the mixtures used for active immunization. A mixture that is only slightly toxic to the guinea pig gives the most satisfactory results in non-immunes. Von Behring did not give in detail the exact proportions of toxin and antitoxin. He injected neutral and slightly toxic mixtures. The doses he recommended were only a fraction of the ones we used.

Dr. Zinsser: We are learning all the time that we can immunize with antigen-antibody combinations in which apparently no free antigen is present. This is, of course, the principle underlying immunization with sensitized vaccines as introduced by Besredka and the early experiments of Kraus and others on protection with sensitized cholera spirilla. It seems hard to understand this unless we assume dissociation of the antigen-antibody complex within the body.

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FOUR CASES OF PRIMARY CARCINOMA OF THE LUNG

ROBERT A. LAMBERT, M.D.

(*From the Pathological Laboratory of Montefiore Hospital, New York City*)

The relative infrequency of primary carcinoma of the lung is the reason for bringing before the Society four specimens which were encountered among less than fifty autopsies performed at Montefiore Hospital during the past eight months. Such a high incidence is very unusual and is undoubtedly accidental, in view of the reports of incidence of 0.2 to 1.0 per cent. from many other institutions. A review of Dr. Adler's excellent monograph on "Primary Malignant Growths of the Lungs and Bronchi," in which all of the published cases have been collected, has convinced me that our four cases belong to well recognized types of lung carcinoma. The first two are distinct squamous celled epitheliomata, prickle cells and keratinizing "pearls" being frequently encountered. The third belongs to the group of adenocarcinomata arising in bronchial glands. The cells exhibit in many places an active secretion of mucus. The fourth is a papillary adenocarcinoma, the cells of which possess in places definite cilia.

An interesting feature of these cases is the difference in the tendency toward metastasis formation. The first two tumors showed no metastases. The third, on the other hand, besides an extensive local spreading, metastasized to the axillary, cervical and abdominal lymph nodes, and liver; while in the fourth case, a papillary ciliated epithelial tumor, metastatic nodules were present, not only in most of the abdominal viscera, but also in various bones (vertebræ and ribs especially) and in the brain.

The squamous cells of the first two tumors, which are generally thought to arise from the cylindrical epithelium of the bronchi, must be regarded as having strayed far from the parent type; that is, the cells are, in this sense, very atypical. Yet, in their behaviour they appeared less malignant than the more nearly typical cells of the third and fourth cases. Indeed, it was the ciliated epithelial cells of the last case that manifested the most pronounced tendency to metastasize.

Complete reports of these cases will be published elsewhere.

Discussion:

Dr. Norris: I was greatly interested in Dr. Lambert's presentation. He has been very fortunate in finding four cases of primary carcinoma of the lung in such a small series of autopsies. At Bellevue we have records of approximately 4,800 autopsies, and I have seen but two cases of supposed primary tumor of the lung. One of these cases occurred in October, 1904. Recently in looking over the records of primary carcinomas of the lungs, the protocol of the case does not mention the condition of the œsophagus. The tumor was a frank epithelioma. I question very much that the case represents a true primary epithelioma or carcinoma of the lung. The second case occurred more recently. There was a large tumor mass at the hylus of the lung, involving the bronchial and tracheal lymph nodes; there were no metastases with the exception of a small nodule in the small intestines. The microscopic picture in both cases was similar, namely, the tumor was acinous in structure with production of mucus. I do not feel sure that the small nodule in the intestine was not the primary seat of the growth. This autopsy was done very carefully and it was thoroughly sifted.

Dr. Schultze: I held an autopsy on one of Dr. Adler's cases where there was a terminal hæmorrhage. I took issue with the doctor as to its being an endothelioma. I considered the case one of carcinoma of the lung. Since then I have seen two cases which may be primary endothelioma. One started in the bronchial lymph nodes with metastases into the bone marrow. Probably both types of tumors exist.

Dr. Lambert: I think Dr. Norris's criticism is pertinent. It is possible that one of these cases may not be primary in the lung. There was a small erosion of the œsophagus. But I do not think the tumor could have been an œsophageal growth. It would be remarkable for a tumor of the œsophagus to infiltrate the lung as extensively as this one did. I do not think there is any doubt about the other three tumors being primary in the lung. The autopsy was complete in each case and a careful search was made for other possible sites of origin.

REPORT OF AN UNUSUAL CASE, PRESENTING PRIMARY, MULTIPLE, BENIGN, MEDULLARY, GIANT-CELL BONE TUMORS

H. S. MARTLAND, M.D.

(From the Pathological Laboratory, Newark City Hospital)

Introduction

Sarcomata occurring in or near the ends of long bones, endosteal, medullary or myeloid in character, and showing a microscopical picture characterized by numerous giant-cells, having abundant, dark staining and often vacuolated cytoplasm, numerous and regular nuclei with absence of mitotic figures, and a stroma consisting of fibroblastic granulation tissue without active mitoses, dilated and engorged blood-vessels, hemorrhage in perivascular tissue, erosion and disintegration of bone trabeculae have been recognized for many years under such terms as medullary or myelogenous giant-cell sarcomata.

Adami and Bland Sutton have objected to their classification as sarcomata on the grounds that the type of giant-cell encountered in this tumor is a cell resembling the osteoclast or myeloplax occurring in normal bone marrow, and also, from the well known clinical fact, that these tumors were much less malignant than periosteal sarcomata, and in many cases distinctly benign.

Bloodgood, in 1902, was the first in this country to seriously study this tumor, and he concluded that he did not believe that this type of tumor ever caused metastases, but would recur *in loco* when not thoroughly removed, a fact that was recognized as early as 1895, by Koenig, Bergmann and Mikulicz. Bloodgood therefore concluded that it was not a sarcoma, and for a better name, termed it medullary giant-cell tumor.

Mallory considers the giant-cells encountered in this tumor as foreign-body giant-cells, formed by the fusion of endothelial leucocytes, and believes that in forming a diagnosis, they should be considered as entirely of secondary value. Mallory would attempt to trace the tumor back to its type cell, say the fibroblast, and the tumor's malignancy would be determined by the presence or absence of active mitosis.

Barrie, in 1913, called attention to the characteristic macroscopic and microscopic appearances of these tumors, which are entirely different from that of malignant periosteal tumors. Barrie believes that the process is not a tumor formation at all, but merely a chronic non-suppurating form of osteomyelitis, therefore he has named the lesion chronic non-suppurative hemorrhagic osteomyelitis.

The table below gives a condensed idea of the clinical and pathological facts known, in so-called medullary giant-cell sarcoma or chronic hemorrhagic osteomyelitis of Barrie.

CLINICAL FACTS.

Situation: Ends of long bones, especially upper end of tibia.

Trauma: Definite history in large majority of cases.

Age: Young people, large majority.

Onset: Usually sudden.

Malignancy: Will recur *in loco* after incomplete removal, but will never form metastases.

Roentgenograms: Expansive, abrupt and circumscribed tumors; distention may be great, bone shell preserved.

PATHOLOGICAL FACTS.

Gross Appearance: Tumors usually confined within periosteum. Definitely circumscribed, not infiltrating. Easily removed from bone shell. Are distinctly vascular, resembling young granulation tissue, friable, soft and ooze. Resemble currant-jelly or fresh cut liver. Later this picture may change, and the tumor become cystic.

Microscopic Appearance: Giant-cells of foreign-body or osteoclastic type.

Nuclei: Regular in size and shape. Numerous. Absence of mitotic figures.

Cytoplasm: Dark staining. Abundant and often vacuolated.

Stroma of Tumor: Suggests young granulation tissue, with endothelial-lined blood-spaces separated by connective tissue stroma, filled with spindle and round cells, showing few if any mitotic figures.

I wish to present the histories, clinical courses, and pathological findings in two cases presenting bone tumors of the above described type. Both patients are from the surgical service of Dr. F. R. Haussling at the Newark City Hospital

The first case presents an expansive tumor involving the lower end of the right tibia, of over eight years' duration, which on microscopical examination shows the typical picture of the so-called medullary giant-cell sarcoma. I present this case to demonstrate the benign character of this well-known tumor.

The second case presents multiple expansive bone tumors, the clinical picture resembling that of multiple myeloma; the microscopical appearance of the tumors, however, is that of the so-called medullary giant-cell sarcoma. Multiple tumors presenting this microscopical appearance have hitherto been undescribed.

CASE I. *History:* A. L. Male, Italian; age twenty-eight years; barber. Past history: Had chancre when eighteen years old. In 1907, he twisted his right ankle while playing ball; leg was put in plaster three weeks; he then received luetic treatment for one month; plaster cast again applied for two months; lower end of tibia curetted, and after wound healed he was discharged as cured. In 1908, the swelling and pain returned in the ankle; the tibia was again curetted, a diagnosis of osteomyelitis made and he was discharged with a persistent sinus. In 1910, he fell and again injured ankle; he was at this time admitted to the City Hospital. Subsequent history: The lower end of the tibia was curetted, and microscopical examination showed myelogenous giant-cell sarcoma. The wound healed, but old sinus persisted. During 1911, the tibia was curetted four times for recurrence. In 1915, the tibia was again curetted for recurrence in and about sinus, and the surgeon noted that the original bone cavity was no longer filled with red granulation tissue, but consisted of several cystic cavities filled with clear fluid. Between these last curettages, he has been able to work as a barber, and his general physical condition is excellent.

Summary: The points of interest in this case are:

(1) A single, expansive tumor of the lower end of the tibia.
(2) Slow growth, over eight years' duration.
(3) Eight curettages for recurrence, which is always *in loco*.
(4) Absence of metastases.
(5) Excellent physical condition of patient.
(6) Typical gross appearance of tumor is changing from that of a cavity filled with red granulation tissue (chronic hemorrhagic osteomyelitis of Barrie) to that of bone cysts filled with clear fluid (chronic fibrocystic osteomyelitis of Barrie).
(7) Typical microscopical appearance of the so-called medullary giant-cell sarcoma or chronic hemorrhagic osteomyelitis of Barrie.

CASE II. *History:* T. K. Female; age twenty-five years; married; housewife. Family history: Negative. Past history:

Had measles in childhood; venereal diseases denied; has had four normal births, one miscarriage. In February, 1914, patient had a miscarriage; she lost considerable blood and has never been well since. She complained of weakness, dyspnœa, and palpitation on exertion. In August, 1914, she noticed a lump growing in the inner angle of right eye; this was the first tumor mass noticed. In October, 1914, she fell and broke right femur about six inches above the knee-joint. She was admitted to the City Hospital and the fracture united with good result. In February, 1915, she noticed a mass on the anterior surface of the left tibia, about its middle third, which grew for two months and attained the size of a hen's small egg; it then stopped growing. In May, 1915, she gave birth to a full term, healthy child. In June, 1915, she was admitted to the City Hospital, complaining of dyspnœa, weakness, cardiac palpitation and slight bone pains.

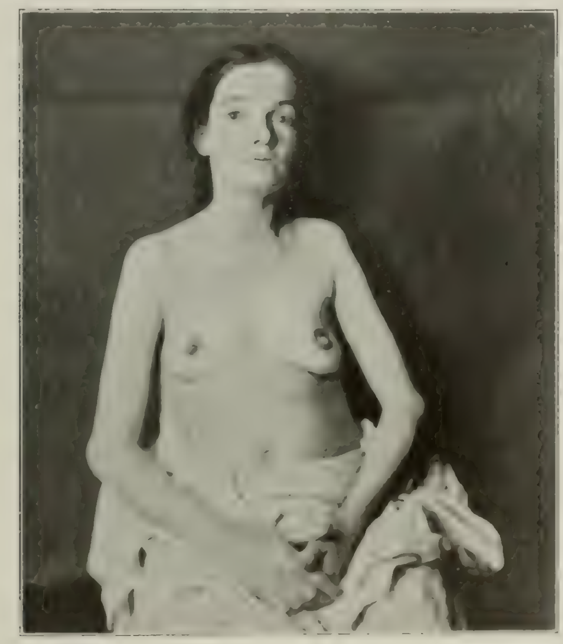

FIG. 1. Photograph of multiple bone tumor case, showing growth in right antrum.

Physical Examination: A well-developed female, fairly well nourished (Fig. 1). Nose, saddle shaped (due to trauma in childhood). Heart, apex in sixth interspace, outside nipple line; a loud mitral systolic murmur. Lungs, suspicious signs of early tuberculous involvement of left upper lobe. Rest of physical examination negative, except for the presence of palpable bone tumors. The right superior maxilla presented a hard tumor mass, which was infiltrating the inner wall and floor of the right orbital cavity. The left clavicle presented a hard mass the size of an English walnut at the sterno-clavicular joint. The right clavicle near its acromial end presented a hard tumor mass the size of an English walnut. The left tibia, on its anterior surface, and near its middle and upper third, presented a hard tumor the size of an English walnut.

Roentgenograms: Showed in addition to the palpable tumors, described above, expansive tumors in the following locations: In the seventh rib on the right side; in the right femur above the knee-joint (Fig. 2); in the right fibula on its upper third; and in the left fibula on its upper third (Fig. 3). Later roentgenograms showed a tumor developing in the right humerus just above the elbow (Fig. 4), and in the pelvis.

Laboratory Examinations: The following laboratory examinations were made:

Hæmoglobin	75 to 85 per cent.
Erythrocytes	4,020,000 (none abnormal)
Leucocytes	5,200 to 9,600 (none abnormal)

Blood Wassermann negative, three times
Spinal fluid Wassermann, negative once, with normal cell count and globulin

Urine: Several 24-hour specimens were examined, especially for Bence-Jones albumose, with negative results. The urine always showed a trace of albumin, with a small number of hyaline and granular casts. Otherwise the urine was normal.

Clinical Diagnosis: From the above clinical facts a diagnosis of multiple myeloma, cardiac dilatation, and failing compensation was made.

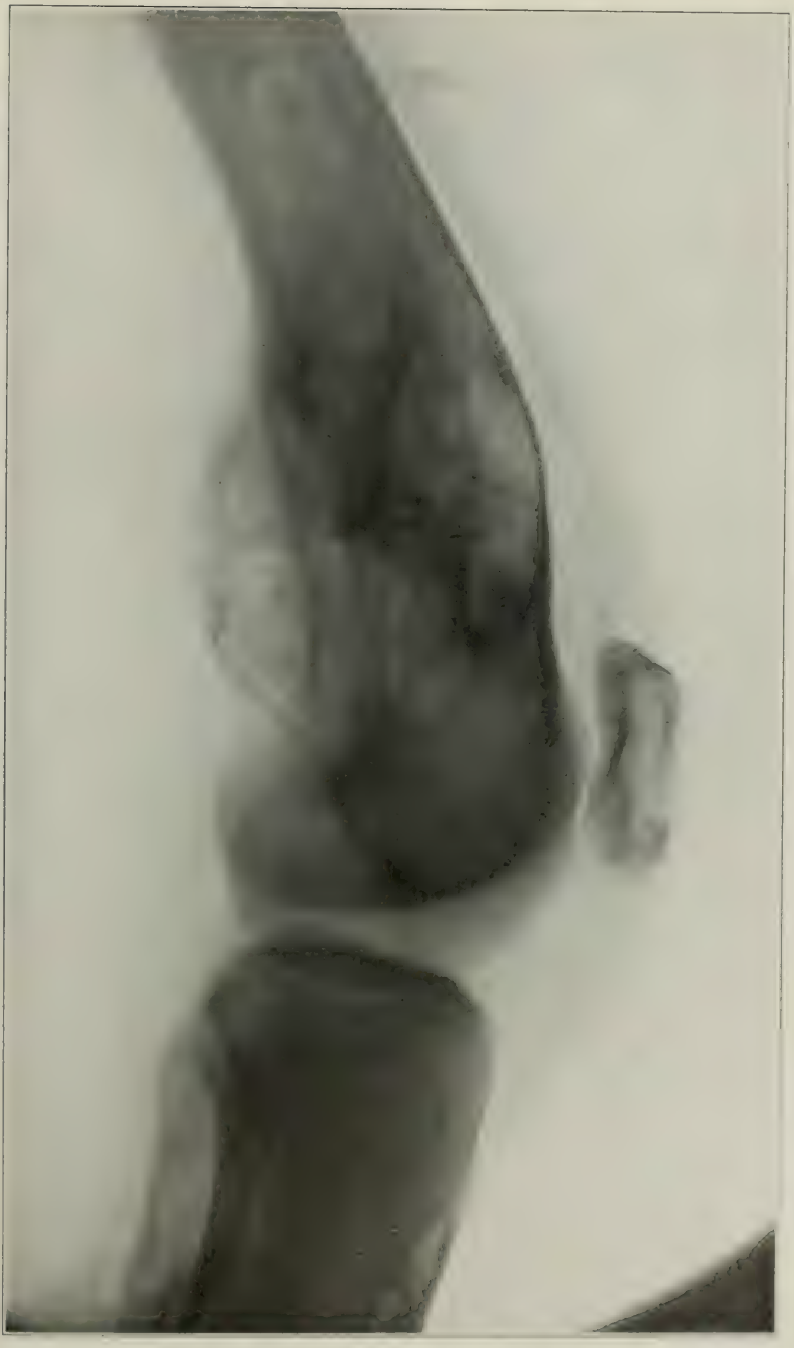

FIG. 2. Roentgenogram, showing expansive growth in lower end of right femur.

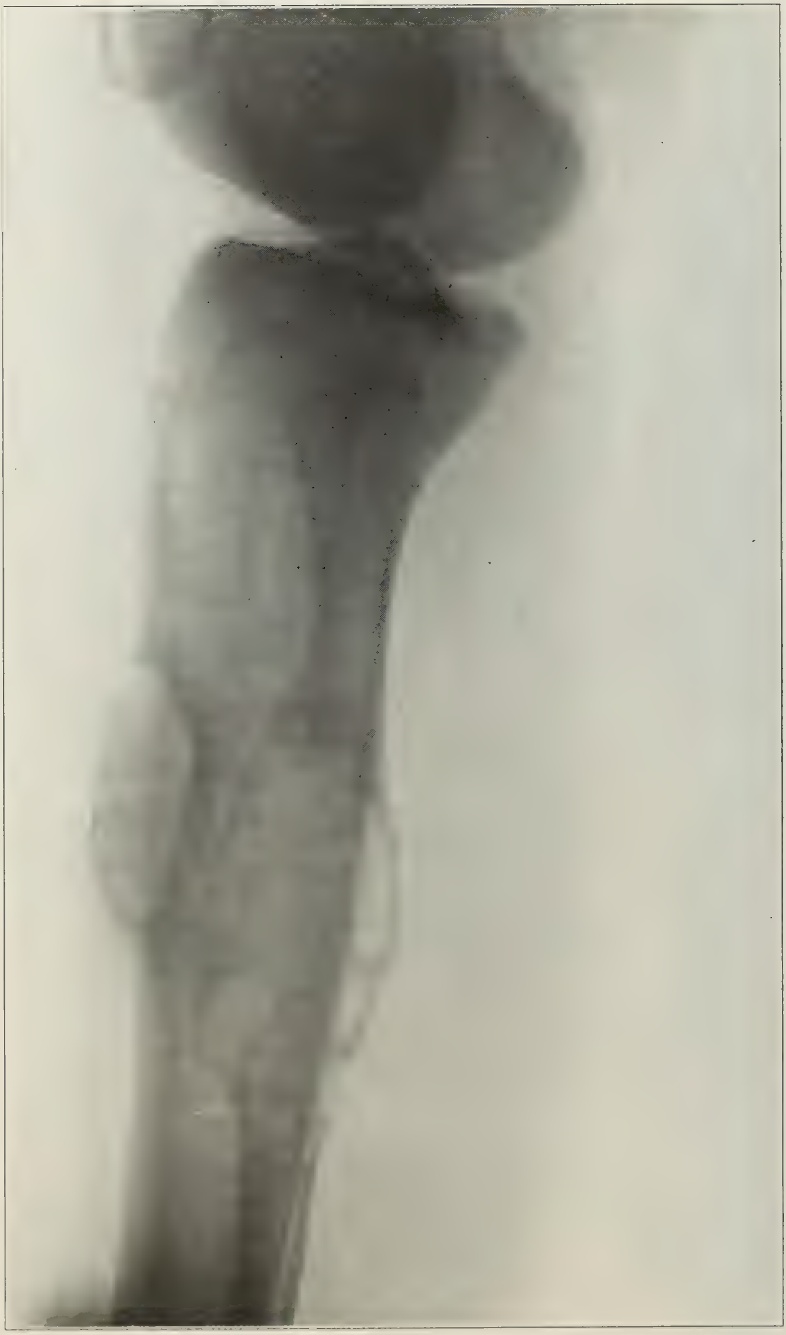

FIG. 3. Roentgenogram, showing expansive growths in left tibia and fibula.

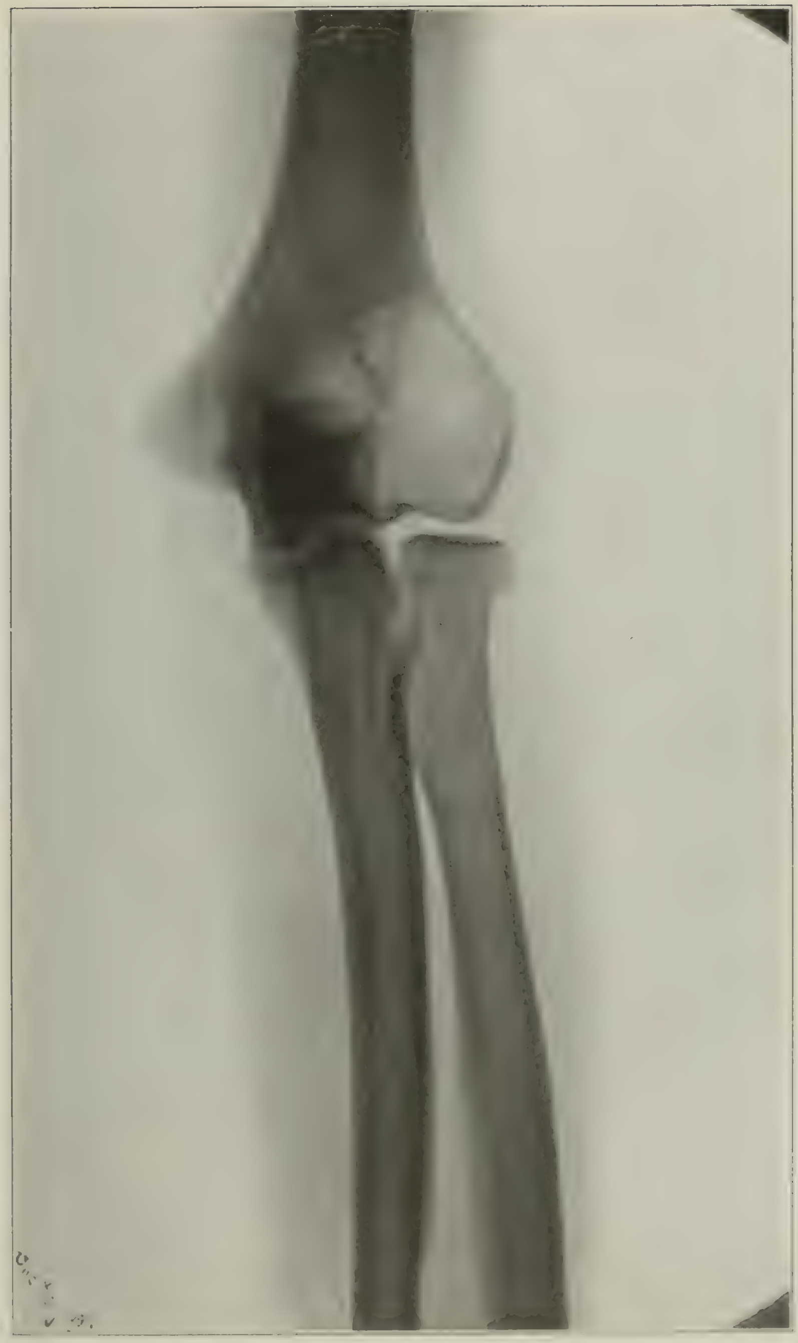

Fig. 4. Roentgenogram, showing expansive growth in lower end of right humerus.

Progress Notes: On June 28, 1915, in order to establish a positive diagnosis, Dr. Haussling curetted the growth on the left tibia. At the operation, he added the following notations to the history: "Incision revealed a small localized cavity, containing what appeared to be well-organized granulation tissue, reddish, and with considerable bleeding. Tissue curetted away, cavity swabbed with pure carbolic." Wound healed by first intention.

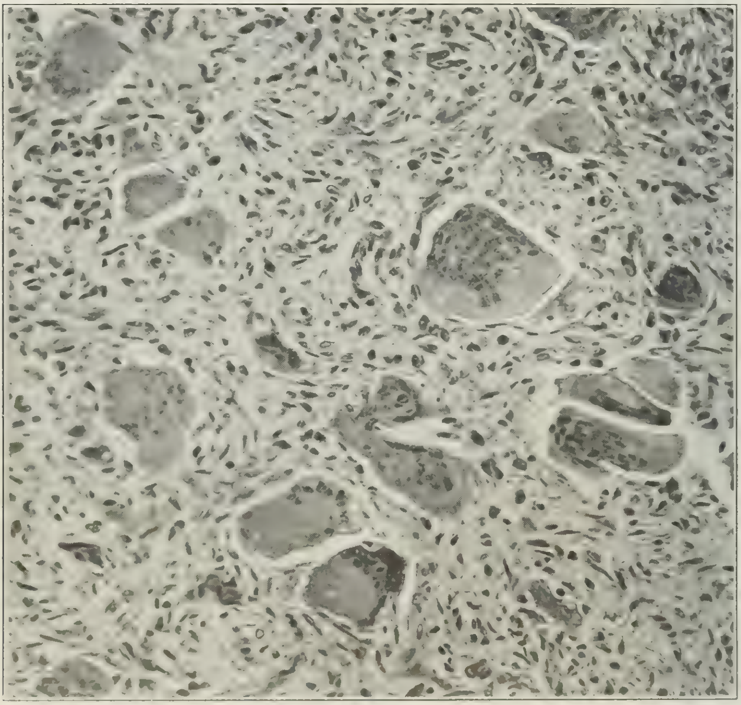

Fig. 5. Case II. One of the multiple tumors. Showing numerous giant-cells with fibroblastic stroma.

On microscopical examination of the curetted tissue, I was surprised to find the typical lesion of the so-called myelogenous giant-cell sarcoma. Believing this to be a benign lesion from our previous experience, Dr. Haussling decided to attempt to remove by the curette all the other tumor masses. On August 16, 1915, a second operation was performed and tumor masses in the following locations were exposed and curetted: right fibula; left fibula; left clavicle; seventh rib and right superior maxilla. The following notation was made by Dr. Haussling at the operation: "The lesion in the superior maxilla seems to involve the entire antrum and to be encroaching on the right nasal cavity. All tumors are well localized, the line of demarcation of diseased and healthy bone is quite sharp. The gross appearance is that of reddish, pale granulation tissue with considerable bleeding." All incisions healed by first intention. Microscopical examination of the various tumor tissue removed at the operation showed the typical picture of the so-called myelogenous giant-cell sarcoma (Figs. 5 and 6).

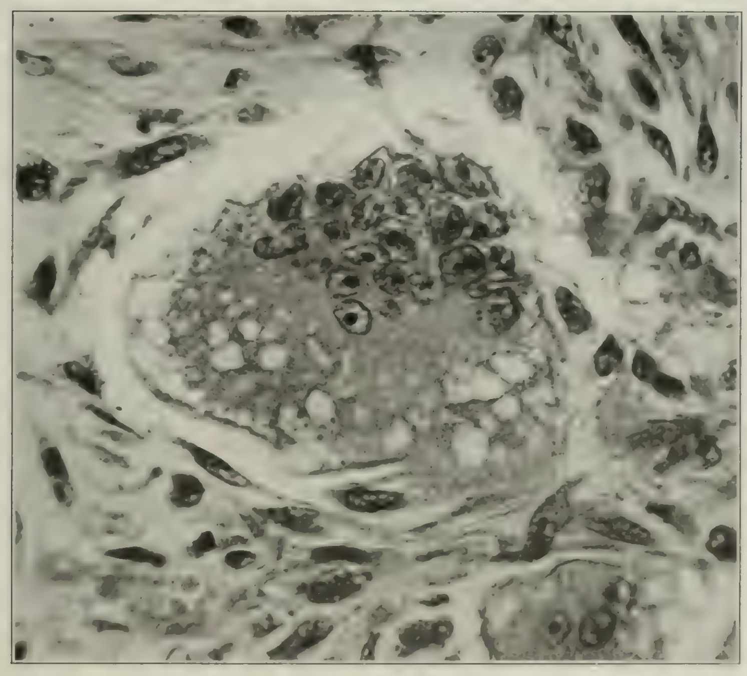

FIG. 6. Case II. High-power picture of typical giant-cell. Showing dark, abundant cytoplasm with numerous regular nuclei without mitotic figures.

Subsequent Notes: The patient's physical condition has greatly improved; her cardiac condition has become fully compensated. The tumors curetted still show in roentgenograms, and new ones are slowly making their appearance.

Summary: The points of interest in this case are:

(1) Multiple, primary, expansive, slow growing bone tumors.
(2) Tumors too numerous and situation too varied to infer trauma as an etiological factor.
(3) Tumors are well localized and circumscribed.
(4) There is absence of infiltration of soft parts by any of the tumors, in fact, none of them have yet broken through their bony shell.
(5) There has been very little bone pain.
(6) There is absence of visceral or other metastases.
(7) There is absence of emaciation, in fact the physical condition improves.
(8) All incisions over tumors healed by first intention, a condition not often seen in malignant bone tumors.
(9) All tumors had a typical or characteristic macroscopical appearance, namely showing solid and cystic portions, the former predominating, and resembling reddish granulation tissue with free bleeding.
(10) All tumors presented the typical microscopical picture of the so-called medullary giant-cell sarcoma or the chronic hemorrhagic osteomyelitis of Barrie.

Discussion:

Dr. George Barrie: In cases of hæmorrhagic non-suppurating osteomyelitis, or the so-called giant-cell myelogenous sarcoma that I have seen, the roentgenograms were different and did not resemble those shown by Dr. Martland from the multiple bone tumor case. As regards Mallory's views, I do not think the instances he has reported should be regarded as typical.

Dr. Coley: In any discussion pertaining to the question: whether myeloid tumors are benign or malignant, one must first determine what is meant by the term "myeloid sarcoma." One surgeon will call the small and sharply defined non-infiltrating tumors of the bones "malignant or round-celled sarcoma," while most of these are benign; and another includes in the term "myeloid sarcoma" all endosteal tumors, many of which are of extreme malignancy. One of the most important discussions of Tumors of the Long Bones was held at the Royal Society of Medicine in London (November, 1912). In the course of the discussion, Sir Frederick Eve stated that it was an error to consider all myeloid tumors as benign. Mr. Gilbert Scott, of London, stated that while the majority were benign some of them were not only malignant, but showed a very marked degree of malignancy. Mr. R. C. Maybury cited two cases of myeloid sarcoma, observed at St. Thomas' Hospital, both of which were followed by general metastases which proved fatal.

The second case reported by Dr. Martland this evening had none of the clinical features of ordinary myeloid sarcoma of the long bones, nor did the x-ray picture point to such a condition. Dr. Coley stated that he believed the nearest approach to such a case as the one just observed was a case of Dr. Gibney's, in which slow-growing tumors developed in one long bone after another (usually followed by spontaneous fracture) until a large number of the bones were involved, finally causing death. The x-ray picture of these lesions is shown in Dr. Coley's recent paper on "Some Problems in the Early Diagnosis and Treatment of Sarcoma of the Long Bones" (*Annals of Surgery*, November, 1914).

Dr. Coley stated that he did not agree with Dr. Bloodgood and some others, who regard all cases of myeloid sarcoma of the long bones as benign. In his paper, referred to above, may be found a microscopical photograph of a tumor which occurred in the upper part of the humerus, in a boy ten years old. The growth attained its largest size three weeks after the injury. The clinical symptoms in this case pointed so clearly to a highly malignant sarcoma that Dr. Coley decided to perform immediately an amputation at the shoulder-joint, without waiting for a microscopical section. This he did, and later submitted a specimen of the tumor to Dr. Ewing, who pronounced it a giant-celled sarcoma of the epulis type showing only a moderate degree of malignancy. Dr. Ewing believed that Dr. Coley had made a mistake in amputating in a case that he thought could have been cured simply by curetting. In spite of the very radical operation, which was followed by a short course of toxin treatment, extensive metastases developed in the lung and the boy died within a year.[1]

Dr. Coley showed x-ray photographs of two cases of central sarcoma, recently observed at the Hospital for Ruptured and Crippled, one occurring in the humerus and the other in the tibia; both were classified by the pathologist as giant-celled sarcoma. Nevertheless, the clinical course of the disease proved it to be of a high degree of malignancy. In the first case there was extensive invasion of the knee-joint by a tumor of eight months' duration, and the whole lower end of the femur was destroyed. In the second case, the whole upper end of the femur was destroyed and extensive involvement of the fibula had taken place within seven months.

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[1] It is only fair to state that on later examination of the specimen by Dr. Ewing he inclined to the belief that the tumor belonged to the type of bone aneurism, of a very high degree of malignancy, and that his original diagnosis was undoubtedly erroneous.

Dr. Lambert: I was very much interested in the second case of Dr. Martland. I would like to ask if any other such cases have been reported. Regarding Dr. Coley's remarks, he tells us that he has seen examples of a typical giant-cell myeloid sarcoma of a highly malignant character. I wish he had brought sections from those tumors. I am inclined to think that he has confused the giant-cell myeloid sarcoma with the mixed sarcoma of bone, in which multinucleated cells are found. The latter tumor is, however, quite different from the former, both clinically and pathologically. The former is characterized not only by the presence of numerous large giant cells, but by an abundant connective tissue.

To regard the giant cells in myeloid sarcoma as accidental foreign-body giant cells, as Mallory does, appears to me to be without basis. Their formation is too uniform and constant to admit such an interpretation. Furthermore, foreign particles are not found in these cells.

Dr. Martland: In the multiple case the clinical appearance is like multiple myeloma. Histologically we find it resembling the single tumor case, therefore I was led to believe that these multiple growths were of the same nature and that they were benign in character. I do not believe that the tumors in this case will ever metastasize into the soft parts. The case will be carefully followed and will no doubt come to autopsy, and nothing will ever be satisfactorily determined until then. I think the antrum tumor will grow so large that it will invade the ethmoid cells and cause a meningitis, but I do not think she will ever die of metastases. I can find no evidence in literature of any case of multiple tumors of slow growth which have the same histological picture. As far as hypernephroma is concerned, we do not think it necessary to consider that in this case.

EQUILIBRIUM IN THE COMBINATION AND THE DISSOCIATION OF PRECIPITATES

RICHARD WEIL, M.D.

It has been shown by Linossier and Lemoine that if horse serum or other similar antigen be mixed with its specific precipitating anti-serum, the resulting precipitate never exhausts completely either of these two factors. Both of them can always be shown to be present in the supernatant fluid. This has been confirmed by Eisenberg and others. Moreover, the serum of immunized animals has been shown under certain circumstances to contain both precipitin and precipitinogen. By the original observers this was interpreted as an instance of the laws of mass action. Zinsser and Young interpreted it on the analogy of the protective action of a third colloid on two mutually precipitating colloids. Von Dungern attributed the observation to the presence of a multiplicity of antigens in the horse serum, or similar antigenic substance, with a corresponding mutiplicity of antibodies. The whole phenomenon, upon this basis, is reducible to a fallacy of technique. This theory was not supported by experiment and has not found further acceptance.

In order to test this theory a chemically pure antigen was prepared, namely crystalline egg albumen. When this is mixed with its anti-serum a precipitate results. The supernatant fluid can always be shown to contain either one of the two factors, egg albumen or antibody, but never both at the same time. This is true no matter how the proportions may be varied. With raw egg albumen and its anti-serum, on the other hand, the relations are the same as described by Eisenberg. The conclusion is drawn that Von Dungern's theory is correct. Under proper experimental conditions the precipitation reaction goes on to the complete exhaustion of either factor. "Equilibrium does not exist."

By means of absorption experiments it was shown that raw egg albumen contains not only the substance isolated as crystalline egg albumen, but also other antigens. Likewise, the anti-serum to raw egg albumen was shown to contain a mutiplicity of antibodies. This confirms the previous conclusion.

In view of the fact that equilibrium in precipitation reactions does not exist, it is unnecessary to consider the explanations offered therefor. It is of interest to note, however, that the presence of either small or large amounts of serum, such as rabbit serum or guinea-pig serum, added to the above mixtures, does not in the slightest interfere with the completeness of the reaction. There appears, therefore, to be no analogy in this instance with the protective action of a third colloid.

Dissociation of precipitates has recently been studied by Chickering in connection with an extract of pneumococcus and its anti-serum. He used salt solution, or a one per cent. solution of sodium carbonate. He found that the extract contained no antigen, but did contain protective antibody and agglutinin. In my own experiments in dissociating the precipitate produced by horse serum, or egg albumen, and its appropriate anti-serum, different results were obtained. If the precipitate was treated with salt solution or sodium carbonate, antigen could always be demonstrated in the extract. It contained no precipitin, but did contain antibodies conferring passive sensitization. If the extraction was performed with trypsin or with rabbit's leucocytes, both antigen and precipitin could be demonstrated in the fluid. These reactions, like those previously described, have no analogy either in mass action or in colloidal chemistry.

Discussion:

Dr. Field: I would like to ask Dr. Weil how far digestion was carried with the leucocytes and trypsin. In Osborne and Mendel's work, when they carried digestion to completion there was loss of toxicity. This is used in testing the activity of proteolytic enzymes. In regard to the sodium carbonate and sodium chloride it might be due to precipitation.

Dr. Zinsser: In a matter of this kind time is needed for the discussion. I do not think it is logical, on the basis of these observations, to minimize the analogy between colloidal chemistry and serum reactions. In the precipitin reaction we have the influence of salts which is a striking analogy. We have the inhibition zones and the influence of quantitative relations on the reaction. We are dealing with obscure phenomena and must call analogy to our help and we have a right to reason from it, and the analogy to colloidal chemistry is too valuable to be disregarded. Five or six years ago Dr. Young and I were working on this problem. We did not consider the explanation of Von Dungern as likely. In our work we found that when we allowed the serum to stand it would precipitate spontaneously. The antibodies in the supernatant fluid would correspondingly diminish. This we thought was a reasonable argument that the antibody antigen complex came together by slow union. This, too, is analogous to colloidal suspensions where slow precipitation occurs even in the presence of a protective colloid. The mass action idea we did not consider correct because the sera themselves gave no complement fixation. This test is not absolute proof but it is the only indicator available. If the mass action idea was correct, then the serum itself should have contained antigen-antibody united as well as dissociated, and this complex, as far as we have any reason to argue from experience, should have fixed complement. I think that the precipitation phenomena of serum are too difficult at present to throw out peremptorily any help we get from colloidal analogy. So far as separation of the anaphylactic antibody from the precipitins goes, I may say that I have been one of those who for a number of years have maintained, both in experiment and in reasoning from the work of others, that there is only one antibody of which precipitation, agglutination, and other antibody reactions are different manifestations, depending upon the conditions under which the reactions are carried out and the nature of the antigen.

DR. FIELD: In regard to the question of colloids and mass action, I used to use the term colloid chemistry, but I am sure we have got to be on our guard in making use of these names. I think the types of reactions between ions of extremely small size and molecules of larger size, and passing to molecules of organic compounds and then to colloidal gold and finally to clay and carbon, differ only slightly from each other.

DR. ZINSSER: I think that what Dr. Field says is true, and that no one has thought of disputing the perfectly self-evident statements he has made. That we are dealing with larger particles in one case and with smaller ones in the other, and, in the case of the larger particles, often with substances that are not chemically analyzable, is perfectly true, but it is also true that the methods of study of these two different kinds of substances are necessarily different, and since we believe that antigen-antibody reactions are of the latter nature, we apply these latter methods to their study.

DR. WEIL: The remark has just been made that the argument from colloidal reactions is really only a "cloud to cover our ignorance," and I think this remark truly conveys the gist of the whole matter. Instead of solving the problems of immunology we have been using the analogies of colloidal chemistry to veil our ignorance of the situation. I believe that the dangers and fallacies of such a course are evident from the results of the experiments which I have described this evening. The actual results of the immunological experiment do not in the least correspond to the analogy which had been drawn from the reactions of colloidal chemistry. This danger was long ago emphasized by Buxton, and even by Bordet, the arch-exponent of the colloidal interpretation. As regards the particular questions brought up, the problem of auto-precipitation would require too long a time for discussion here. I may say, however, that I do not agree with Dr. Zinsser's interpretation of this phenomenon. A full discussion of that phase will be presented in a publication of mine which will soon appear. There is another point in my paper to which Dr. Zinsser took exception, namely, the question of separation of the precipitin from the anaphylactic antibody. He feels that they are the same substance. I entirely agree upon that point. The two functions, however, namely, precipitation and passive sensitization, may be differentiated and separated. I have found that it is possible by heating precipitin to 72 degrees to deprive it completely of its precipitating effect, while the sensitizing value is retained almost unimpaired. In the terminology of Ehrlich the so-called ergophore group of the antibody, which is essential to precipitation, may be sacrificed, while leaving intact the so-called haptophore group, which alone is necessary for anaphylaxis. This observation, which is, I believe, of great importance for the understanding of anaphylaxis, has not been hitherto reported. It answers Dr. Zinsser's objection satisfactorily.

GROUPING OF MENINGOCOCCUS STRAINS BY MEANS OF COMPLEMENT FIXATION

MIRIAM P. OLMSTEAD

A study of literature on the meningococcus shows that while the complement fixation method has been used extensively in titrating serum and to a slight extent for diagnosis, except for work with the para-meningococcus practically no attempt has been made to use it in grouping strains of meningococci.

The work I am to report this evening has been done in collaboration with Dr. Phoebe DuBois, Dr. Josephine Neal and Miss Rose Schweitzer.

To obtain immune serum rabbits were inoculated by ten intravenous injections of live culture, administered every third day. The dose was increased from one fourth to a whole slant culture, washed off with normal saline. The cultures for inoculation were twenty-four hour growths in neutral veal agar.

Antigens were prepared according to the method suggested by Schwartz and McNeil for gonococcus antigen.

The total volume of the test was 0.5 c.c., one tenth that of the classical Wassermann. The anti-sheep system was used, cells in a five per cent. suspension, from one to two units of amboceptor, and guinea-pig serum for complement in a ten per cent. dilution. The system was carefully standardized each day by an amboceptor titration. Sensitized cells were used and the readings of antigen and antibody content titrations were made the next day, after the cells had settled.

The sera were freshly inactivated each day by heating for one half hour at 56° C.

Antigen and serum titrations were performed according to the technique described in Park and Williams' *Pathogenic Microorganisms*, 1914, pp. 184 187. The anticomplementary dose of antigen has been determined by testing decreasing amounts of antigen (0.3, 0.2, 0.1, 0.05 c.c.) in various dilutions. The antigen unit, by which is meant the smallest amount of antigen that gives complete inhibition of hemolysis with about two units of homologous immune serum, has been obtained by testing decreasing amounts of antigen (0.25, 0.2, 0.15, 0.1, 0.05, 0.025 c.c.), with 0.01 c.c. of serum. The anticomplementary property of the serum has been tested in 0.04 c.c. and 0.02 c.c. of undiluted serum. The antibody unit, by which is meant the smallest amount of serum that gives complete inhibition of hemolysis with about two units of homologous antigen, has been obtained by testing decreasing amounts of serum (0.02, 0.01, and of a ten per cent. dilution 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01 c.c.), with about 0.1 c.c. of antigen.

The work on the differentiation of meningococcus strains was begun with neither antigen nor serum standardized. A polyvalent antigen of all the strains was made and standardized with 0.01 c.c. of serum from a horse immunized against all the strains. The polyvalent horse serum was then titrated with one and one half units of the polyvalent antigen and used in standardizing all the monovalent antigens. Each monovalent antigen was titrated in several dilutions to obtain the antigen unit. From one and one half to two units of the monovalent antigen thus standardized were used in standardizing its homologous immune serum. Any antigen so poor that double this amount was anticomplementary was discarded and not used for serum titrations. Serum was also titrated in a sufficient number of dilutions (1 in 10, and 1 in 100) to determine the antibody unit. From one and one half to two units of serum (diluted so that 0.01 c.c. contained that amount) were used in making the antigen cross-titrations. The specificity of the antigens was tested by titrating against a known positive gonococcus serum with practically negative results.

Rabbits were immunized against forty strains of meningococcus, but the cross-fixation work was carried out completely with only twenty-nine of these strains. Each antigen and each serum were titrated against all the strains in several dilutions until the smallest amount of both antigen and serum that gave complete inhibition of hemolysis was determined.

The results of antigen titrations are summarized in the table. Of the twenty-nine strains of meningococcus tested, fourteen fell into one group, eight into another, two cross-fixed with each other only by both antigen and serum titrations, two did not fix with any other strain and three acted irregularly. Strains 1, 3, 4, 7, 8, 19, 20, 21, 22, 23, 24, 26, 28, and 34 cross-fixed with each other, forming Group I (Tables I and II). Strains 9, 10, 11, 13, 14, 16, 18, and 32 cross-fixed with each other, forming Group II (Tables I and II). Antigens of Group I did not fix

TABLE I

Antigens of Twenty-two Strains Titrated Against Sera of Various Strains

Antigen Strains	Immune Sera of Group I									Immune Sera of Group II						Immune Sera of Irregular Strains			Immune Sera of Odd Strains			
	1	3	4	7	20	22	23	24	28	10	11	13	14	16	18	2	12	27	6	29	15	17
1	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
3	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
4	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
7	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
8	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
19	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
20	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
21	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
23	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
26	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
28		+	+	+	+	+	+	+	+	—	—	—	—	—	—		—	—	—	—	—	—
34		+	+	+	+	+	+	+	+	—	—	—	—	—	—		—	—	—	—	—	—
9	—	+	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	±	—	—	—	—
10	—	+	—	—	—	—	±	—	+	+	+	+	+	+	+	—	—	±	—	—	±	—
16	—	+	—	—	—	—	—	—	±	+	+	+	+	+	—	—	—	—		—	—	—
18	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	+	—	—	—	—
32	—	—	—	—	—	—	—	—	—	+	+	+	+	+	+	—	—	—	—	—	—	—
2	+	+	+	+	—	+	+	+	+	—	—	—	—	—	—	+	—	—	—	—	—	—
27		+	+	+	—	+	—	—	+	—	—	—	—	—	—		—	+	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—
29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+

+ Denotes complete inhibition of hemolysis in as small an amount of antigen with heterologous as with homologous serum.

± Denotes complete inhibition of hemolysis, but not in so small an amount of antigen with heterologous as with homologous serum.

— Denotes incomplete or no inhibition of hemolysis.

any sera of Group II, but antigens 9, 10, and 16 of Group II fixed some sera of Group I. Antigen titrations only of strains 21 and 26 were made against other single strains, as the rabbit

TABLE II

Immune Sera of Twenty-four Strains Titrated Against Various Antigens.

Serum Strains	Antigens of Group I											Antigens of Group II						Antigens of Irregular Strains			Antigens of Odd Strains		
	1	3	4	7	8	19	20	22	23	24	28	9	10	13	14	16	18	2	12	27	6	29	15
1	+	+		+	+	±	±	+	±	±	±	−	−	−	−		±	−	±	−	−	−	−
3	+	+	+	+	±	±	+	+	+	+	+	+	+	+	+		±	+	±	+	−	−	−
4	+	+	+	+	±	−	+	+				−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	−	−	+	+	+	+		−	−		−		−	−	−	−	−	−	−
8	+	±	+		+		+	+				−	±	−	−		−	±	±	−	−		−
19	+	+	+	+	+		+					+	+		−		+	±	−		−	−	−
20		+	+	+	+	−	+	+				−	−	−	−	−	−	±	−		−		
22	+	+	+	+	±	±		+				±	±	±	±		±	±	±		−		
23	+	+				+	+					±	±	±			±				−	−	
24	+	+		+		+	+					+	+				±		+	+	−	−	
28	+	+		±				+				+	+		−		−	+			−	−	−
9	±	±	±	±	±			±	±			+	+	+	+			±	±		−		
10	±	+		±	±			−				+	+	+	+	+	+	±	±	−	±	−	
13	±	±	±	±	±			±				+	+	+				±	±		±		
14	±	±	±	±	±		+	±				+	+	+	+	+	+	±	±	+		−	−
16	±	±	±	−	−	−		±				+	+			+	+	−	−		−		
18	+	+		+	+		−					+	+			+	+	−	+		−		−
2	−	−	−		−	−		−				−	−		−	−	−	+	−	−	−	−	−
12	±	±	±		±							±	±		−			±	+		−	−	±
27	±	−	±	−			±					±	±			±	±	−		+	−		−
6	±	−	−	−	−	−	−	−	−	−	−	−	−	−				−	−	−	+	−	±
29	−	−	−	−			−					−	−		−		−	−	−		−	+	−
15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−	+
17	±	±	±	−	−	−	−	−	±	−	−	−	−	−	−		−	−	−	−	−	−	+

+ Denotes complete inhibition of hemolysis, in approximately the same amount of serum with homologous and heterologous strains.

± Denotes complete inhibition of hemolysis, but not in so small amount of serum with heterologous as with homologous strains.

— Denotes incomplete or no inhibition of hemolysis.

sera of these strains lost their antibody content before serum titrations could be performed. These strains were not included in an antigen made of six strains (3, 21, 10, 18, 2, and 12). The sera of these strains (21 and 26), however, gave fixation with this polyvalent antigen and the antigens of these strains fixed all the sera of Group I, hence, we concluded that these strains belonged to Group I. Antigens of strains 6 and 29 gave fixation with their homologous sera only and sera 6 and 29 gave fixation with their homologous antigens only. Antigens and sera of strains 15 and 17 cross-fixed with each other. Strains 2, 12, and 27 acted irregularly. In the antigen titrations of these strains cross-fixation occurred with the sera of Group I, but the immune sera of strains 2, 12, and 27 fixed complement with their homologous antigens only.

Sera of twelve strains (1, 3, 4, 7, 8, 19, 20, 21, 23, 24, 26, and 28) of Group I, seven strains (9, 10, 11, 13, 14, 16, and 18) of Group II, the irregular strains 2, 12, and 27, the odd strains 6, 29, 15, and 17, and nine unidentified strains (31, 32, 33, 34, 36, 37, 38, 39, and 40), were titrated with a polyvalent antigen consisting of strains 3 and 21 of Group I, 10 and 18 of Group II and the irregular strains 2 and 12. The relationship of strains 6, 15, 17, 27, and 29 had not been determined at the time this antigen was made or they would have been included. These strains only gave no fixation with the antigen, but our later work demonstrated that they were not related to any of the strains included in the antigen.

As stated above, the monovalent antigens were standardized with the polyvalent immune horse serum for the serum cross-titrations, which were made before the antigen cross-titrations. We found after we had done the antigen cross-titrations, that the antigens gave a much longer range of fixation with their homologous immune rabbit serum than with the polyvalent horse serum. It seems, therefore, that so much antigen was used in the serum cross-titrations that a group reaction occurred. This may explain why a more distinct differentiation of strains was obtained by means of antigen titrations than by means of antibody content titrations.

Complement fixation is such a delicate method of differentiating strains of an organism that it is difficult to obtain absolutely consistent results. The age and antibody content of a serum, the range of the antigen, the strength of the complement, the interval between the completion of the test and the reading of the same, the temperature of the ice-box, and the frequency of standardization of sera and antigens are factors that greatly influence the test.

A study of the history of the strains proves that the clinical history of the case has nothing to do with the grouping.

Strains causing severe reactions in rabbits—it being necessary to try several rabbits before one could be found that could be immunized—were:

1, 2, 7, 16, 18, and 24,—
1, 7, and 24 are in Group I,
16, and 18 are in Group II, and
2 in irregular Group.

Thus it appears that no relation exists between virulence and grouping. Neither is there any relation between length of time strains had been isolated and grouping. Group I contained the oldest and the newest strains.

The subject of the para-meningococcus has received considerable attention in France for the last few years, due to the work of Dopter, Menetrier and others. This work has been done along both clinical and laboratory lines. Patients suffering from meningitis that did not respond to the ordinary anti-meningitis serum improved under the use of a para-meningitis serum prepared by Dopter in 1912. Dopter in 1911 in studying the so-called para-meningococcus, found that he could not differentiate these strains from normal meningococcus by using them as antigens with polyvalent horse serum, but that they could be differentiated by the serum of patients, as the serum of patients suffering from meningococcic meningitis did not fix a para-meningococcic antigen, and the sera of patients suffering from para-meningococcic meningitis did not fix with a meningococcic antigen.

Wollstein in 1914 published a very careful study of the para-meningococcus in which she arrived at similar conclusions using the sera of rabbits immunized with single strains of meningococcus and para-meningococcus instead of the sera of patients. She concluded: "The para-meningococci of Dopter are culturally indistinguishable from true or normal meningococci, but serologically they exhibit differences as regards agglutination, opsonization, and complement deviation."

By the agglutination method strains 7 and 42 are classed by her as para-like strains. She did not give the results of complement fixation with these strains. In our work it was found by complement fixation that strain 7 fell in Group I and strain 42 in the irregular group allied to Group I. As transplants of two Dopter para-meningococcus strains, P. L. and P. M., were kindly given us by Dr. Wollstein, we were enabled to test them against our strains. We found antigens of P. L. to react with homologous serum only, and antigens of P. M. to react with the sera of Group II.

Now if the para-meningococcus is to be separated from the normal meningococcus by serological reactions and if agglutination and complement fixation yield different results with the same strains, the question is indeed a perplexing one. With the method used by us, the results yielded by the complement fixation test have been fairly uniform and specific. By this method the strains have fallen into various groups, containing a varying number of strains. Shall we call all the strains except those in Group I para strains, or shall we call them simply groups of normal meningococci? The question is purely academic, yet it is of some importance since the employment of the term "para" differentiates the strains more than seems justifiable. It is perhaps better, therefore, to classify them as belonging to a group co-ordinate with other meningococcus groups.

Wollstein further concludes: "Because of the variations and irregularities of serum reaction existing among otherwise normal strains of meningococci it does not seem either possible or desirable to separate the para-meningococci into a strictly definite class.

It appears desirable to consider them as constituting a special strain among meningococci not, however, wholly consistent in itself." This conclusion we would heartily endorse.

CONCLUSIONS

By means of complement fixation the meningococcus may be clearly differentiated from allied organisms. A differentiation of individual meningococcus strains is possible by the use of refined technique, but the relationship of strains is so close that it is difficult to obtain absolutely clear cut and consistent results. Of the twenty-nine strains studied by us, fourteen seem to form one group and eight a second group. Three seem to be closely related to the first group but have acted so irregularly that they cannot be classed with it, two have shown a relationship with each other only and two have shown a relationship to no other strain.

Discussion:

Dr. Zinsser: The difficulty of separating and classifying the various strains of meningococci is well known. This is the first serious attempt to clearly separate the different strains by complement fixation tests.

THE PRACTICAL SIGNIFICANCE OF THE OXIDASE REACTION AS APPLIED TO BLOOD CELLS

F. A. EVANS, M.D.

For some time it has been known that the cells of myeloid origin contain granules of oxidase ferment and those of lymphoid origin do not, and an appreciation of the practical significance of this difference has led to many attempts to perfect a convenient method of demonstrating the presence or absence of the ferment in any given cell. As early as 1887, Vitali made the observation that the guaiac test for blood was of no value in the presence of pus, but Brandenburg[1] in 1900, was the first to notice that blood of leukemic patients frequently gave a positive guaiac reaction without the presence of any oxygen bearer as peroxide or ozonized turpentine. Meyer[2] in 1903, demonstrated this phenomenon in the blood of myeloid leukemia but failed to obtain the reaction in that of lymphoid leukemia and called attention to the interest that attached to this reaction in view of Ehrlich's recently propounded dualistic theory of blood cell origin. This guaiac test was too crude to be of any practical value however, so Winkler[3] in 1907, applied the indophenol-blue synthesis of Ehrlich[4] (alphanaphthol + dimethylparaphenylendiamin with two atoms of oxygen form indophenol-blue) to smears of pus and tissue sections and with it found these granules of oxidase ferment as blue-black dots in the protoplasm of myeloid but not of lymphoid cells. He at this time predicted the usefulness of this reaction in the study of the origin of white blood cells, and it is the practical value of this test that we have under consideration this evening.

Schultze[5] submitted sections of many body tissues to this reaction after formalin fixation and found the granules only in the tear and thyroid glands and in cells of myeloid origin, and used the test in the differential diagnosis of the leukemias. Gierke,[6] using fresh tissue, found these granules in many body cells but after formalin fixation as used by Schultze obtained similar results. He suggested the hypothesis that this reaction on fresh tissue depended on the circulating respiratory oxygenase destroyed or inactivated by formalin fixation whereas the more stable oxidase of the myeloid cell endures. This work of both Schultze and Gierke has been repeatedly confirmed by other observers.[7] Gierke also refuted Dietrich's[8] statement that the oxidation is brought about by the tissue metabolism and the preformed indophenol-blue taken up by the fat droplets, by showing that no granules appear after killing the ferments and adding the already oxidized solutions, and that granules still give the reaction after killing the cell life by chloroform but preserving the oxidases. This indophenol-blue reaction appears, therefore, to be a reliable method, but although much superior to the guaiac test, because of technical difficulties is not all that is to be desired.

For fixation, only methods are available that do not injure ferments and as shown by Gierke,[6] formalin must be used in some form. Similarly counterstaining is difficult for only aqueous solutions used without a mordant are permissible, and any method of differentiating destroys the reaction in some cells. Furthermore, inasmuch as this indophenol-blue deposited in the granules of oxidase ferment is soluble in alcohol, ether, chloroform, aceton, xylol, and creosote, clearing of tissue sections is impossible and the mounting media are almost limited to materials soluble in water. Also, the preparation is not permanent, for the oxidase reaction only endures in its original intensity for about an hour, and the counterstain tends to be dissolved out by the aqueous mounting medium.

In spite of these difficulties, methods of application have been devised that are of practical value for tissue sections and especially for smears of blood and body fluids. For tissues, paraffin sections may be used if fixed primarily in formalin or preferably Bonn solution (Carlsbad salts 5, formalin 12.5, water 100 parts) as suggested by Fursenko,[9] for the materials used in preparation presumably do not injure the ferments. Frozen sections freshly cut give reliable results more constantly and are to be preferred. The section is immersed for about two minutes in a mixture in equal parts of the reagents freshly made up:

(1) 1 per cent. alpha-naphthol in 1 per cent. KOH.

(2) 1 per cent. aqueous dimethylparaphenylendiamin.

These solutions should be mixed just before using for if they are allowed to stand together for any length of time they combine with the oxygen of the air with the formation of indophenol-blue and confusing artefactions are likely to occur if granules of this preformed indophenol-blue are deposited on the section. The tissue removed from this mixture is washed, counterstained for a few moments in 5 per cent. pyronin or saturated aqueous safranin, washed again, and mounted in glycerin or a sugar mixture. Such a preparation shows the nuclei stained a deep orange-pink and the myeloid cells standing out by reason of the blue granules in their protoplasm. However, owing to the impossibility of clearing the section satisfactorily and the somewhat indefinite counterstaining, reliable identification of the individual cell as polymorphonuclear, myelocyte, or other, is frequently difficult. For this purpose parallel sections must be stained with hematoxylin and eosin and corresponding fields compared; or the field showing oxidase-containing cells may be drawn with a camera lucida, and the same section then stained with hematoxylin and eosin. If a frozen section has been used, after removing the cover slip and washing, the section is best dried and fixed to the slide with thin celloidin solution. Without these auxiliary procedures, the reaction is of value in demonstrating mild grades of infection in cellular tissues as the appendix and tonsil, and in demonstrating myeloid infiltration of organs in leukemia; and with them may be used to advantage in studying any obscure body cell. Many modifications of the indophenol-blue reaction have been suggested by Loele,[10] Schultze,[11] Kreibich,[12] and others,[13] whereby permanent preparations that may be cleared and counterstained may be had, but in all of these so many other factors are to be considered that their specificity for the myeloid cells may be seriously questioned. These modifications, although of great interest in the field of vital staining,[14] do not displace the original indophenol-blue reaction.

For smears of blood and other body fluids a satisfactory and more direct technique is available. Fixation is the most important step and for this, exposure to formalin vapor in a closed jar is the only satisfactory method. Very weak solutions of formalin (4 per cent.) for long periods (3–4 hours), depending on the age and thickness of the smear, are better than using stronger solutions for shorter periods, for with the weaker solutions the annoying tendency of the fixed smear film to wrinkle up when the indophenol-blue solutions are added, is not so marked. For smears, in contrast to tissue sections, the staining is best done first and for this the same solutions, 5 per cent. pyronin or saturated safranin, have proved the most satisfactory. Either of these allowed to remain on the smear a short time and then thoroughly washed off brings out the nuclei a deep orange-pink that readily admits of the identification of the cell and leaves the other elements unstained. Such a preparation is then examined until a field containing cells in question as regards their oxidase content is found, and the slide clamped down. One drop of each of the solutions of the indophenol-blue reaction made up as outlined above is then added directly to the smear and the progress of the reaction watched with the low power objective in the cells being investigated till it has reached its maximum intensity in about two minutes. The smear may then be blotted carefully and the preparation examined with the high dry objective. It is essential that the cells in question be located before putting on the solutions of the indophenol-blue reaction and that the progress of the reaction be watched, for the stain is rapidly dissolved out of the nuclei by the alkaline solutions used and shortly after adding, especially if some wrinkling of the film as frequently occurs is present, identification of the cells becomes impossible. But if these details are observed a useful preparation results which shows oxidase granules in polymorphonuclears, myelocytes, myeloblasts, transitionals, and probably some large mononuclears, but in no lymphocyte. As in tissue sections the reaction is of value in the study of cells of uncertain identity and is especially useful in the differential diagnosis of the acute leukemias which is so difficult by the usual staining methods. The intensity of the reaction varies directly with the stage of maturity of the cells, being weakest in the myeloblasts and strongest in the polymorphonuclears. One observer[15] suggests that granules of oxidase ferment may not be demonstrable in very young myeloblasts. The eosinophile cells both in blood and tissues show the most active oxidases.

Owing to technical difficulties the indophenol-blue reaction falls somewhat short of the promise it gives at first thought, and modifications devised to obviate these difficulties are not trustworthy. But the methods outlined above, if followed in detail, afford a reaction on tissue sections that is of some value, and one on blood smears, that by reason of the ready differentiation between cells of lymphoid and myeloid origin made possible is of real diagnostic importance.

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Discussion:

Dr. Lambert: In two cases of myeloblastic leukemia recently observed in the Presbyterian Hospital, this reaction proved very valuable. Under casual examination I am certain that the cells in the blood would have been called lymphocytes. In fact, this mistake was made. The oxidase test proved conclusively, however, that the cells were of myeloid origin.

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DEMONSTRATION OF THREE CASES OF SPLENIC ENLARGEMENT

MORTIMER WARREN, M.D.

These three specimens represent different types of splenic enlargement, and are presented to bring up the question of clinical differentiation, and pathological classification of splenomegalia in general, with reference to therapeutic splenectomy.

Case I.—The spleen is from a patient presenting a well-characterized clinical condition with striking differential diagnostic signs, namely constitutional hæmolytic icterus. This spleen was removed by Dr. Charles H. Peck in May, 1912. A complete symptomatic cure resulted, namely relief of jaundice, and a reversal of a previously highly decreased osmotic tension of the red blood cells to one within normal limits. Histologically it is typical of that condition. The spleen is suffused with blood; this blood is chiefly interstitial; there is little or no fibrosis; follicles are somewhat crowded out. In other words, it is a spodogenous spleen tumor very similar to that of pernicious anæmia.

Case II.—The spleen represents a type which has many designations but which seems to belong to the Banti group. Though still "*sub judice,*" as an entity, Bänti's disease has a place in any discussion of splenic enlargement. There is a group of cases in which clinical results are obtained by splenectomy which cannot be listed elsewhere except under the general terminology of "splenic anæmia." This spleen shows marked diffuse fibrosis without a great deal of capsular thickening, obliteration of follicles, and thickening of arterioles. There is little blood or congestion. (A small piece of liver removed at the time of the operation appears normal on section.) Splenectomy was performed by Dr. Charles H. Peck, March, 1914. The man was in good condition when last heard from, within a few months of the present time.

Case III.—The patient showed an enlarged liver and spleen, jaundice, urobilin and bilirubin in urine, and clay-colored stools. There was nothing important in the blood findings aside from the auto-agglutinating property of the blood serum for the red blood cells (brought out only in the cold). Splenectomy was done by Dr. Charles H. Peck, September 15, 1915. Results so far are indefinite. The spleen shows wide open sinuses, areas of distended capillaries, abundant follicles, fibrosis of a fairly young character (cellular). The spleen looks as if it were in a hyperactive state, analogous to a lymph node in a state of irritative hyperplasia. A small bit of liver removed at the time of operation showed inflammation of a subacute character. There are periportal and scattered areas of round cell and polynuclear infiltration with some peri-portal increase of connective tissue. A diagnosis of peri-portal pylephlebitis was made.

I should like a discussion, especially in regard to the condition of the spleen accompanying cirrhosis of the liver, the spleen of malaria, and of syphilis.

Discussion:

Dr. Norris: These cases are of great interest. Much importance has been attached to the spleen in regard to its association with various diseases. I do not feel able to give a competent opinion upon the lesions of the spleen. It has always been a subject of great dispute among pathologists. Even the histology of the spleen has not been definitely settled. The lesions that Banti has described as characteristic for the disease known by his name, namely, fibro-adenia, I have found frequently present in the spleen where Banti's disease has not been present, namely, in association with many of the conditions found in middle life and also with recessive status lymphaticus. It is quite frequent to find thickened or nearly obliterated arteries of the splenic follicles, and often there are marked fibrous changes not only about the vessels but in the stroma. I cannot say, therefore, that these changes are characteristic of any one disease and therefore not characteristic of Banti's disease. A number of pathologists have described the spleen as presenting characteristic appearances not only in the gross but on microscopic section; this has not been my experience. In syphilitic cirrhosis of the liver, the enlargement of the liver is often marked and it is well known that the size of the spleen apparently bears no especial relation to the degree of the cirrhosis of the liver.

Dr. Larkin: We are in a hopeless condition, it seems to me, in regard to the classification of splenic lesions. I feel Dr. Norris is right. There are so many allied conditions which bring enlargement of the spleen which have no special characteristics, that we are hopelessly involved. Dr. Warren's specimen shows well marked enlargement and engorgement of the spleen in the thrombosis of the splenic vein. We do not know any more than we did many years ago about the exact condition of the circulation of the spleen, histologically.

Dr. Levy: What was the red cell count in these cases?

Dr. Warren: None of the three cases showed any anemia of any account.

Dr. Levy: Was there polycythemia?

Dr. Warren: No.

Dr. Zinsser: Did I understand Dr. Warren to say that one of these cases had hæmolysins for its own cells?

Dr. Warren: There were auto-agglutinins in the third case brought out only in the cold.

Dr. Zinsser: These conditions seem to be analogous to paroxysmal hæmoglobinuria.

Dr. Warren: I don't know what it means. There did not seem to be direct clinical indication for operation, except on the basis that the spleen had an important bearing on the liver condition and might keep up the disturbance in the liver.

Dr. Zinsser: In paroxysmal hæmoglobinuria an autolysin is found which does not act under the ordinary conditions of incubation. Hæmolysis occurs only when the sensitization has occurred at a low temperature and the tubes are consequently warmed up. I should like to ask whether there are any described splenic conditions associated with paroxysmal hæmoglobinuria.

Dr. Warren: I don't know. In these jaundice cases it is associated with the bile production.

WASSERMANN FINDINGS IN LOBAR PNEUMONIA

J. J. HERTZ, M.D.

My communication this evening is a preliminary report on the Wassermann findings in lobar pneumonia. At the Beth Israel Hospital where we do a Wassermann reaction on every patient admitted we found in a series of twelve cases the following results:

I. Ten of the twelve cases got well.

II. Every one of the ten cases that got well gave a "four plus" Wassermann reaction during the course of the disease, between the fifth and eighth day, and a negative reaction after the temperature became normal.

III. Of the two cases that died, one gave a "four plus" reaction on the sixth day and died on the seventh day. On autopsy the organs showed signs of syphilis. The other case that died gave a negative reaction on the eighth day about two hours before death. The only negative case died.

We are carrying on some experiments to find the nature of the substance that gives the reaction in these cases.

We used the original Wassermann test with the exception of the antigen which was an alcoholic extract of guinea pig's heart.

TRANSPLANTABLE SARCOMATA OF THE RAT LIVER ARISING IN THE WALLS OF PARASITIC CYSTS[1]

G. L. ROHDENBURG, M.D., AND F. D. BULLOCK, M.D.

(From Columbia University, George Crocker Special Research Fund, F. C. Wood, Director)

Primary sarcomata of the liver, while not common in man, are relatively frequent in rats, probably because of the relation they bear to infection with the *Tænia crassicola.* In a series of autopsies on rats performed during the past three years, we have encountered three such tumors, two of which were recognizable in the gross, while one was of microscopic size only.

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[1] This report appears in full in *The Journal of Cancer Research*, 1916, I, 87.

The first case has already been reported.[2] The second case occurred in a full-grown male, the tumor being situated in the left lobe of the liver and measuring one centimeter in diameter; metastases were scattered throughout the peritoneal cavity. The neoplasm, a spindle-cell sarcoma, is transplantable, and is now in the tenth generation, with an inoculation percentage of 100. About eighty per cent. of the takes retrogress spontaneously. Attempts to propagate the tumor by using a Berkefeld filtrate and dried tumor tissue were not successful. The third case occurred in an adult female; the tumor was situated in the Spigelian lobe and measured 1.5 cm. in diameter, and there were peritoneal metastases. This tumor also is transplantable, though the percentage of takes is not as high as in the case of the second tumor.

The chief point of interest in these cases is the demonstration of the parasite in the centers of the tumors.

Discussion:

Dr. Zinsser: I would like to ask whether it was at all possible to see where the tumor started.

Dr. Rohdenburg: The tumors started in the cyst wall surrounding the parasite.

Dr. Zinsser: Does the cyst wall originate from the capsule of the liver? Can the cysts be experimentally produced in rats?

Dr. Rohdenburg: Yes, the rats can be infected with the tenia and cysts produced in this way.

Dr. Norris: Was the pancreas tumor an original tumor or a transplant?

Dr. Rohdenburg: It was a metastasis of the original tumor in the pancreas.

Dr. Zinsser: Is the percentage of tenia findings in rats very high; that is, is the percentage of tumors large or small as compared with the percentage of tenia infection?

Dr. Rohdenburg: The presence of the tenia in rats depends chiefly upon the condition in which the dealer keeps his animals. If he changes the drinking water frequently tenia infection is rare; if not, it is common. The tumors occur in only a small percentage of the infected animals.

Dr. Norris: I would like to ask exactly what the sarcomatous process was in the transplants; in other words, what method of injection was used in the transplants.

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[2] *Jour. Med. Research,* 1913, XXVIII, 477.

Dr. Rohdenburg: The material used in the first case was the healthy part of the tumor, about 0.5 cm. distant from the parasite. In the second case, the metastases were used for transplantation.

Dr. Zinsser: You never had metastases in the experimental tumors?

Dr. Rohdenburg: No, not in the transplants.

Dr. Norris: In other words, it is doubtful whether we can call the original tumors sarcomata?

Dr. Rohdenburg: If a malignant tumor can be defined as a collection of cells showing unlimited powers of division, I do not see why the primary tumors should not be called sarcomata. Even the fact that some of the transplanted tumors receded is not an argument against their sarcomatous nature. The Jensen sarcoma recedes in thirty out of fifty transplants; but some of the transplants grow, and produce metastases.

A CASE OF PORTAL THROMBOSIS WITH UNUSUAL COMPLICATIONS

JOHN H. LARKIN, M.D.

Thrombosis of the portal vein is not an unusually rare condition. This will be seen after a perusal of Lissauer's report in *Virchow's Arch.*, 1908, CXCII, 278, which states that among 26,687 cases coming to autopsy a condition of complete or partial occlusion of the portal vein was found sixty-eight times. His very interesting resumé also gives us an idea of the duration of such thrombi in the portal vein and its tributaries, together with the concomitant lesions, such as circulatory ileus, apparent in the most advanced cases. Numerous writers have contributed largely to the pathology of this condition, among whom we might mention Welsh, Versé, Rosenau, Pick, Heller, Lewis, Flexner, Whipple, Winternitz, Kohler, and Chiari. It is now well known that there is a close etiological relationship between portal thrombosis and bacterial infection, and this has been especially dwelt on by Welsh. The primary changes in the intima of the portal vein, together with a slowing of the blood stream, as well as compression of the vein by thickening of its adventitia, pressure of large lymph nodules in the vicinity of the hylus of the liver, or inflammatory adhesions to the wall of the vein are etiological factors which have been well pointed out by many observers. Its relationship with metastasizing tumor, either in the liver itself or in distant parts, blocking the vein with such growth, either directly or by extension into the vein itself, has not been so often referred to in the literature of this condition.

It is interesting to note that in all the cases of portal thrombosis which have been reported the liver itself suffers very little secondarily from such circulatory disturbances as would lead on to infarction.

I wish, however, to present a condition of thrombosis, the etiology of which is foreign to our ordinary conception of the disease. It presents a number of factors of great clinical significance, and on account of the close clinical picture of the individual during life with that of cirrhosis of the liver, I trust that the presentation will be of interest to all.

The history of the individual is as follows:

A negro; forty-five years of age; admitted to City Hospital on February 17, 1915. Died March 30, 1915. Chief complaint was swelling of legs and shortness of breath. Family history: Father died at eighty years, of senility; mother died at sixty-four, cause unknown. Patient was one of fifteen or sixteen children, some of which died in infancy, but the majority are living and well. There was no tuberculosis, cancer, alcoholism, heart, kidney, or venereal disease elicited in the family history. Personal history: Had the ordinary diseases of childhood: measles, diphtheria, typhoid fever, etc. No history of venereal disease obtainable. Occupation was that of a private chef in one of the clubs, where he had been employed for a number of years; he was a mild drinker of beer and whiskey. Three and one half or four years ago patient had an axillary abscess, which was opened.

Last July, while in apparently the best of health, in helping remove a trunk he was thrown down a flight of stairs, a distance of some twenty feet, striking on the trunk at a point on his abdomen midway between the ensiform and the umbilicus in the median line. Upon being picked up and examined it was found that a small hematoma had developed in the rectus muscle, about five cm. below the ensiform. This hematoma was from five to six cm. in diameter. It was not accompanied by any unusual pain at the time, except for skin tension. He was advised by his physician to go home. Late on the same evening, around nine o'clock, he commenced to complain of lancinating, shooting pains in his abdomen, accompanied by a partial collapse and vomiting, the vomitus being a yellow, bile-colored fluid. The pain at this time became so severe that opiates had to be administered. On the following day the patient was no better. He remained in bed for a period of two weeks, during which time the pain had diminished in severity, being deep-seated in character.

In the beginning of August the patient's legs became quite markedly swollen, and in one of his attacks of vomiting he had a hematemesis of bright red blood, jelly-like in consistence and about four ounces in quantity. He was treated by his physician for gastric ulcer.

About the beginning of October his abdomen commenced to swell, but not to such an extent as to make him feel uncomfortable. Swelling of the legs continued and shortness of breath was marked at this time. Examination of his abdomen gave fluid wave by percussion. Patient seemed rather anxious in his manner, and commenced to be emaciated. His color was a light yellowish, which was most marked in the conjunctivæ.

About November 1, patient had a very large gastric hematemesis, losing about a quart of clotted, jelly-colored blood, and at about this time it was noted that his stools were dark and tarry-colored, evidently occult blood. From this time on the patient seemed to become more and more depressed. The swelling of the abdomen became larger and larger, and by the first of December fluid had accumulated to such an extent that it was deemed necessary to tap the patient in order to relieve the fullness of his abdomen. A very large quantity of straw-colored fluid was obtained at this time, after which the patient went into a collapse. Soon after another large hematemesis occurred, which contained many jelly-like clots. The patient's condition at this time was that of a fretful and worried individual. The deep-seated pain now seemed to have disappeared.

The patient remained quietly at home until the first part of February, when he was removed to the City Hospital. Upon admission the patient was found to be a fairly well nourished individual. His abdomen was swollen and both legs were moderately edematous. He complained of a dull, deep-seated pain in his abdomen; his expression was anxious, and he was short of breath. The anemia of the individual was well marked, denoting great loss of blood. On the day of his admission, Feb. 17, patient had another large gastric hematemesis. His stools were tarry-black in color. From this time on until March 30, the patient remained in bed, complaining little, except of abdominal pains and shortness of breath; also of an increasing distress on account of abdominal fluid. On March 10, patient complained so much that tapping was resorted to, and a large amount of straw-colored fluid was withdrawn. On March 27, patient had another large gastric hematemesis, which was repeated on the following day. On March 29, the patient was found in a very weak condition; the abdomen was again commencing to distend. On March 30, patient refused nourishment. He had a final hemorrhage of a large amount of blood containing many clots, and died shortly afterwards. An autopsy was performed on the following day.

The autopsy findings were as follows: Body is that of a fairly well developed colored man about forty-five years of age. There are no physical abnormalities upon external examination.

Heart weighs 500 gm. The left ventricle is dilated. Muscle is pale, friable and flabby. Width of the aorta about the aortic valve is 7 cm. Depth of ventricle is 7 cm. Thickness of the wall of left ventricle is 1.5 cm. While the aorta is slightly thickened, its intimal surface is smooth, except for several slightly raised nodulations, mostly of a fatty atheroma. The pulmonary orifice measures 9 cm. Tricuspid valve measures 13 cm. Mitral valve measures 9 cm. Foramen ovale is closed. Aortic valves are competent; they are slightly thickened along their free edges. The mitral, tricuspid and pulmonary valves show no lesions.

Left lung weighs 500 gm. Organ is bound down in the pleural cavity by adhesions. It is voluminous; contains about the normal amount of pigment. Mucous membrane of the bronchus is pale; there is no thrombus in the pulmonary artery or veins. The lung is air-containing throughout. On section the posterior and dependent part shows edema and a moderate congestion. There is no pneumonia. On section upper anterior lobe is pale and anæmic. There is no edema.

Right lung weighs 600 gm. It is bound down in the cavity by adhesions. Lower base of right lung shows compression atelectasis by fluid in the pleural cavity.

Liver weighs 1,600 gm. Portal vein is thrombosed. Surface of organ is indented and irregular. Consistence of organ is firm. Surface is indented by deep cicatricial scars, bringing about a great deformity of the liver. There is atrophy of the extreme left lobe of liver. The capsule is thickened by an irregular veil-like thickening, which evidently is a distension of the lymphatics. On section, liver shows a rather uniform appearance. Markings are partly obliterated. There is no evidence of connective tissue. The thrombus extends even into the portal radicles. The gall-bladder is enormously distended with bile; no stones.

Spleen weighs 800 gm. Organ shows a chronic interstitial splenitis. Splenic vein at its middle and distal portion is extremely dilated, and at about the junction of the inferior mesentery and the splenic vein there is an aneurismal dilatation at that point in the vein in which there is a parietal, organized thrombus. This has extended up into the portal vein, completely occluding its lumen. It is glistening-white in color, and shows partial canalization.

Pancreas shows extreme atrophy, the normal pancreatic tissue being replaced by fat. There is a supernumerary pancreas.

Left kidney weighs 350 gm. It measures 11 × 6 × 3.5 cm. It is uniformly glazed, and has the appearance of amyloid. The organ does not, however, react to iodin. Markings are obliterated; organ is elastic; has an almost stony hardness; it is not congested. The Malpighian bodies are slightly seen. Capsule of kidney is not adherent, but when pulled off diffuse cicatricial wedges of fibrous connective tissue can be seen. Organ has the appearance of a subacute diffuse nephritis.

Right kidney weighs 325 gm. It measures 11 × 6 × 3.5 cm.

Organ is enlarged; capsule strips with ease. On section it is similar to the left, except that it is slightly more reddish in color. The surface beneath the capsule is not granular. The markings of the kidney are obliterated.

Aorta is slightly thickened; its elasticity is slightly diminished; there is a moderate atherosclerosis, but fatty change in the intima is the most pronounced change. There is no calcification; no evidence of syphilitic atheroma.

Bladder is distended with urine; mucous membrane appears pale, but is normal.

Small intestines are full of blood, which has recently stained the mucosa.

Stomach is dilated and full of blood. It shows a hypertrophic gastritis.

Esophagus is unusually dilated. The venous radicles are enormously distended, and at a point 4 cm. above the pyloric end of the stomach there is an ulcerated area in the mucous membrane leading directly into the esophageal plexus, from which constant bleeding has occurred. Above, at a distance of 13 cm., are three other ulcerated areas, the largest of which is 1 mm.

Anatomical diagnosis: Thrombosis of the portal vein, extending into the portal tributaries and liver; varicosities of the esophageal vein (extreme); multiple rupture of varices; acute diffuse nephritis; chronic hyperplastic spleen; atheroma of aorta; supernumerary pancreas in the duodenum; degeneration of the myocardium; fatty infiltration of pancreas.

Upon further examination of the esophagus, four longitudinal, greatly distended varices take up the lower third of the esophagus; the largest of these measures 2 cm. in diameter. The varices are of unusual dimensions, and many punctate points, filled with thrombi, can be seen.

I thought on account of the enormity and intense dilatation of the varices that it would be a good opportunity for the study of such varices. I therefore placed a cannula in one of the lower varices and washed out with cold water the whole esophageal venous system. I then made a mixture of red oxide of lead and chalk (a mixture which is commonly used in making arterial injections in the autopsy room) and using only slight pressure, filled the esophageal varices, the fluid entering with little or no difficulty. After dissecting out the esophagus, it was placed in cold water until the following day, when an *x*-ray of the distended varices was taken, an illustration (Fig. 1) of which is herewith reproduced.

In a resumé of the literature on thrombosis of the portal vein we find that it is not unusual for this condition to be accompanied by esophageal varices, and that one of the attending clinical features is gastric hematemesis. But in a very careful examination of the literature of such cases no report of a case of portal thrombosis is disclosed which shows so enormous a distention of the esophageal varices or an illness which existed for so long a time as the case which I present to you here to-night. The only case approaching it in this respect was the one reported by Versé, and this, it should be remembered, was some ten years ago. His case is rather unique, and corresponds fairly well with the case presented to you now. Versé, however, gave no measurements of the esophageal distention, nor did he give measurements of the esophageal varices.

I think I am safe in stating that never before has a similar specimen been presented to this Society prepared as this specimen has been prepared, presenting, as it does, a clean-cut anatomical view of the esophageal varices, and showing to what a huge extent such varices may be distended.

The transverse measurement of the esophagus taken at the time of autopsy is 12 cm., and the largest varix, which is almost a venous aneurism, measures 2 cm.

Many punctate points may be seen, from which bleeding has occurred from the distended veins.

A further dissection of the organs reveals the following:

The spleen is enormously enlarged, and on section of the pancreas from tail to head one immediately encounters the distended splenic vein, which, at its middle part, measures 1.5 cm. Just before the splenic vein meets to form the portal there is a giving way of the splenic vein, and an aneurism has formed, the wall of which is now lined by a laminated and organized clot, pearly-white in color, and of firm consistence. From this point on the portal vein is entirely thrombosed, reaching up to the portal radicles. Yet upon macroscopical examination the liver is found to be negative, except that the superior surface of the left lobe seems to be indented by deep cicatricial scars, the apex of which was toward the greater bulk of the liver substance, and

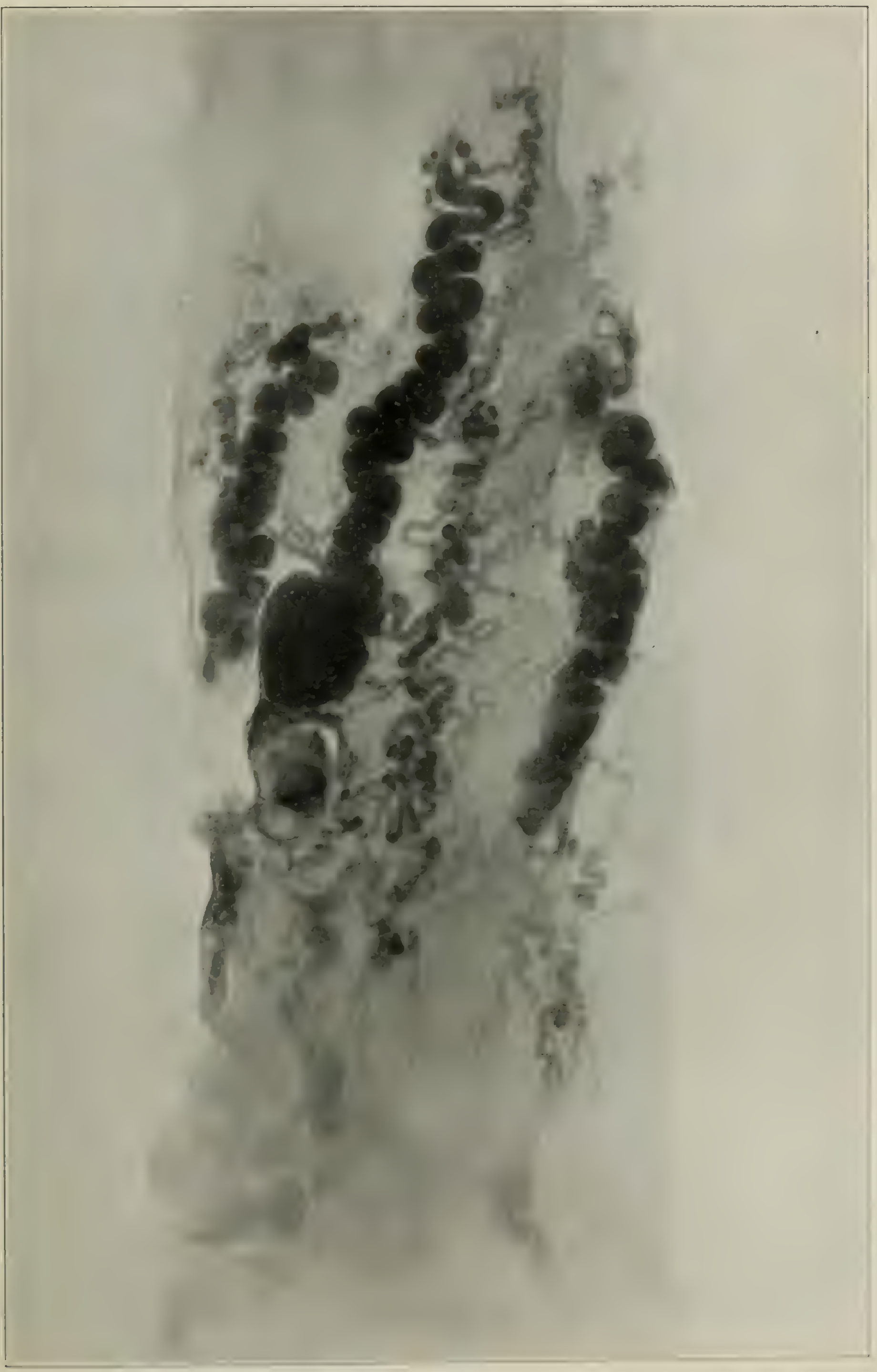

Fig. 1. Excessive Esophageal Varix in a Case of Portal Thrombosis

the base peripherally pulling down and deforming the greater part of the liver tissue. Some of these cicatrices measure 1 cm. from base to apex.

Microscopical examination of the splenic aneurism and portal vein shows a trauma, with resulting aneurismal dilatation in the splenic vein at its junction with the portal. Here the thrombus at its peripheral part is organized, the wall of the vein having given away and showing a much older pigment distributed throughout in the neighborhood of the pancreatic tissue. Examination of the portal vein shows beginning parietal organization, the central part, however, being composed of a firm mixed clot.

Microscopical examination of the liver shows no pathological changes. The cicatrices at the peripheral part of the liver capsule show large triangular areas of dense fibrous connective tissue, in which the hepatic artery and the portal radicles seem to be condensed by such fibroses. No infarction of the liver can be made out either macroscopically or microscopically, and the present interpretation of such cicatricial wedges does not now seem clear to the writer.

This case is of unusual interest, not only to the clinician but to the pathologist, by reason of the very close clinical picture exhibited in portal thrombosis, and disturbances of the portal circulation in cases of cirrhosis of the liver.

It is of far greater interest, however, on account of the etiological factor presented, which is undoubtedly one of trauma—a factor which has not heretofore been mentioned in any report of similar cases. It presents great clinical interest, too, by reason of the enormous distention to which the esophagus may be put before rupture and death intervene. The extent of this dilatation is faithfully shown in the accompanying photograph (Fig. 1).

Discussion:

Dr. Norris: This case of Dr. Larkin's is interesting. In the photomicrograph shown there was evidence of phlebitis. I don't think there is any doubt that trauma was the etiological factor in this case. I notice that the pancreas was large. The relation between latent and sub-latent infections of the pancreas and appendix is interesting. The veins of these organs drain into the portal vein. In view of the trauma in this case, with the damaged vein, there probably developed a lesion of the portal vein which gave rise to thrombosis. In regard to the esophageal dilatation, the thrombus must have been acute and the veins were not able to take care of the distention.

Dr. Larkin: I am glad to hear Dr. Norris has agreed with me in the view of trauma as an etiological factor. I have looked over this subject very thoroughly. In all the cases that have come under my observation the question of trauma has never arisen. I have asked a number of well-informed clinicians but have had no agreement on the etiology of this condition.

BILATERAL INFARCTION OF THE SUPRARENAL BODIES

JOHN H. LARKIN, M.D.

The specimen of infarction of the suprarenal bodies presented to you this evening is so very rare that it deserves more than passing notice. There has been no case similar to it reported in medical literature, save a meager resumé of a unilateral thrombosis of the suprarenal, described by Paul G. Woolley in the *Journal of Medical Research*, 1902. That was a case of an eleven-months-old child, in whom he had found a thrombus of the right adrenal vein, with resulting engorgement and infarction. It is quite well known that hemorrhages in the suprarenal bodies may be unilateral or bilateral, and may be found at the time of autopsy in newborn infants, but to this I do not refer.

The history of the case presented here tonight, in keeping with the findings, is very unusual.

The patient was a young man twenty-four years of age, a muscular iron-worker. He had worked until his admission to the City Hospital on June 16, 1915. His first symptoms were those of pain in the abdomen, in the region of the ensiform. The pain was of a lancinating character, so that opiates had to be administered to control it. The patient's personal and family history were both negative. He was married, and had one child; he denied the use of alcohol and tobacco. A Wassermann test made at time of admission gave a negative result. Temperature upon admission was 101°. A blood culture taken the same day gave negative findings. Patient was hard to control; he wanted to wander around the ward; at times he was doubled up with pains in the abdomen, crying that something be done for his relief. Up to this time no definite diagnosis had been made.

On June 20 patient's pains were slightly decreased; had one vomiting spell; lay rather quietly in bed under opiates.

On June 23 the patient's temperature was 103°, and his abdomen was now tympanitic. Blood cultures taken on June 22 and 23 proved negative.

On June 24 the patient was depressed and had the facial expression of one suffering; the knees were flexed; patient was evidently in a septic condition.

On June 25 blood culture was still negative.

On June 26 patient went into collapse. His abdomen was now distended with gas, and a provisional diagnosis of peritonitis was made.

On June 27 patient went into coma, and died soon after. No surgical operation was made in this particular case.

An autopsy on June 27 revealed the following:

Body is that of a well-nourished and well-developed young man. Rigor mortis is still present; lividity is present on the posterior and dependent portions of body. There are no external physical abnormalities or scars found. Upon opening the abdominal cavity free gas escapes, and the cavity is found filled with fluid pus, yellowish in color. There is about 4.5 liters of this pus. The greater omentum is necrotic, and occupies the epigastric region. The serosa of the intestines is covered with a flocculent, fibrinous exudate. The intestines are adherent to each other. The proximal portion of the appendix is inflamed, and the gland appears necrotic. Upon removing the large and small intestines and separating the adhesions we find in the epigastric region a ruptured ulcer. This ulcer extends to the duodenum, just beyond the pyloric end of the stomach. The thoracic cavity is free of fluid. Base of left lung is adherent to the diaphragm. There is an acute fibrinous pleurisy at base of right lung, which is slightly adherent to the diaphragm.

Heart weighs 290 gm. Pericardial sac contains about 20 cc. of straw-colored fluid. Right side of heart is filled with a chicken-fat clot. Foramen ovale is closed. Mitral measures 9 cm. Mitral valve is normal. Tricuspid valve measures 12 cm. It is likewise normal. Pulmonary valve measures 6.5 cm. There is a slight atheroma just above this valve. The heart muscle on section is beefy-red, firm in consistency, but shows no gross change. The coronaries are normal.

Left lung weighs 550 gm. Pleura is everywhere smooth, except at base, where it is roughened and hemorrhagic. Bronchus is injected, and covered with a serous exudate. Pulmonary vessels are normal. On section lung shows only congestion.

Right lung weighs 600 gm. Externally it is similar to the left. Bronchus of this lung is likewise injected. On section the organ shows only congestion and a moderate amount of edema.

Aorta is normal.

Bladder is normal.

Ureters are normal.

Liver weighs 2,150 gm. On section lobules are indistinct. Liver is the seat of a diffuse parenchymatous degeneration. Gallbladder contains no stones.

Spleen weighs 250 gm. Capsule is thickened, and covered with an organized fibrin. On section organ shows sepsis. The Malpighian bodies are not as distinct as normally.

Left kidney weighs 300 gm. On section organ is the seat of an acute parenchymatous degeneration.

Left adrenal measures 3 x 4 cm. On section it is a deep black in color; consistence is firm, and it is with difficulty that one can distinguish the medullary from the cortical area.

Right kidney weighs 300 gm. On section this organ is similar to the left.

Right adrenal is the seat of a diffuse hemorrhage. Its measurements are the same as the left. On section it presents a condition perfectly similar to the left, the gland being a deep black in color; dry, and on section it is impossible to tell the difference between the cortical and medullary zones. On dissection of the left adrenal vein the lumen is blocked with a mixed white and red thrombus, completely distending its lumen, and stopping rather short in a "finger-gloved" appearance at its entry into the renal vein. A similar thrombus is found in the right adrenal vein, which completely blocks the lumen. This thrombus is firm, red and white in color, and likewise stops short at its entry into the inferior vena cava.

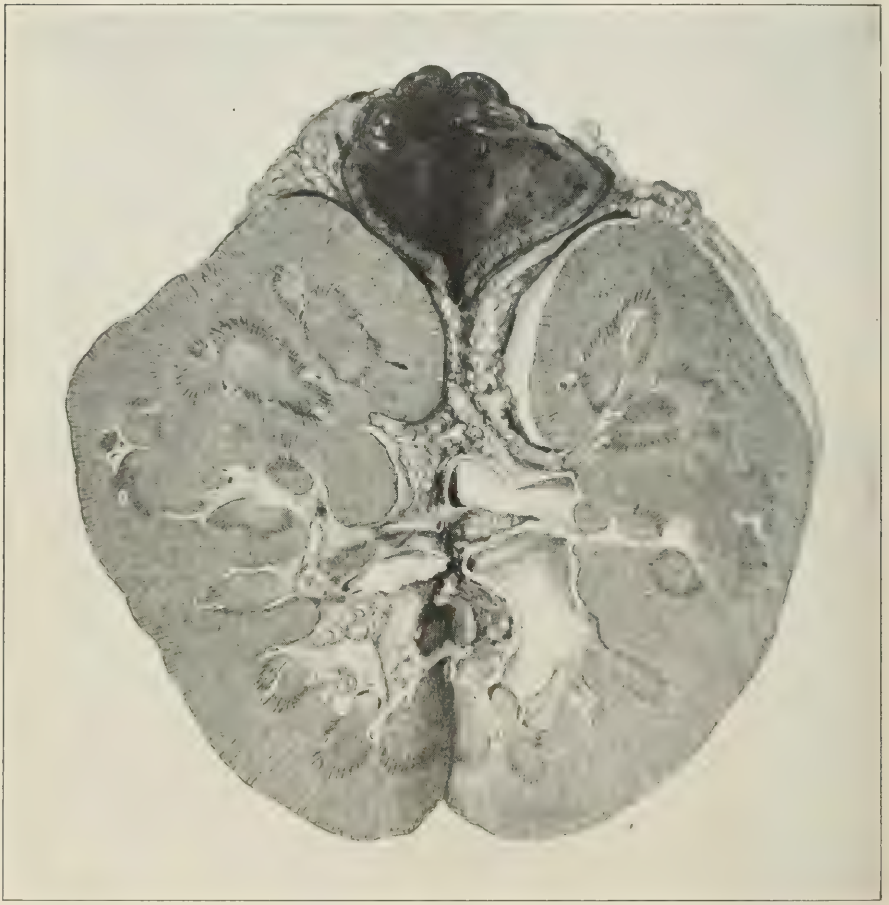

FIG. 2. Hemorrhagic Infarction of Suprarenal in a Case of Perforating Duodenal Ulcer.

Duodenum shows an ulcer about 5 cm. in diameter, which has ruptured into the abdominal cavity.

Anatomical Diagnosis: Bilateral fibrinous pleurisy at bases of lungs; congestion of lungs; septic spleen; cloudy swelling of liver; acute parenchymatous degeneration of kidneys; generalized acute purulent peritonitis, following a rupture of duodenal ulcer; bilateral infarction of adrenals.

This case is one of unusual interest and of great pathological rarity, and only a brief resumé of the condition is presented at this time. It may be well to note that all the writers on thrombosis have failed to describe a condition similar to the one which is presented here in this case. Not even Welsh in his memorable lectures on embolism and thrombosis mentions it, and a close review of the Goulstonian lectures on the suprarenal bodies by Rolleston fails to reveal any mention of this lesion.

The intensity of the infarction is similar in color and consistence to those infarctions that we find in the lung.

The intensity of the infarction is great (Fig. 2) and it may be well to repeat here what Welsh says in his lectures on embolism and thrombosis: "That if the veins are obstructed sufficiently to render the outflow nothing, or exceedingly small, and if the arteries are at the same time open, the infarction is intense."

It is exceedingly difficult to trace the origin of the thrombus in both adrenal veins. Undoubtedly its occurrence in a case of septic peritonitis following a duodenal ulcer which had perforated is a factor. Indeed, one might say that in origin it was probably of bacterial marantic thrombus.

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